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<p><b>(54) Title:</b> FRUIT RIPENING-RELATED GENES</p> <p><b>(57) Abstract</b></p> <p>A vector for use in the genetic transformation of strawberry cells comprises a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region, or a fragment of at least 10 bases thereof, of a strawberry protein selected from O-methyl transferase, acyl carrier protein (ACP), elongation factor, auxin-induced gene, cysteine(thiol) proteinase, cellulase, starch phosphorylase, pyruvate decarboxylase, chalcone reductase, protein kinase, auxin-related gene, sucrose transporter, meristem pattern gene, or selected from a strawberry protein with homology to transcribed sequence accession number T45086, transcribed sequence accession number L36159 or transcribed sequence accession number T45902, or selected from a strawberry protein of unknown homology encoded by one of the StrawRipe sequences A to K.</p>			

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## FRUIT RIPENING-RELATED GENES

This invention relates generally to the modification of a plant phenotype by the regulation of plant gene expression. More specifically it relates to the control of fruit ripening by control of one or more than one gene which is known to be implicated in that process.

### BACKGROUND OF THE INVENTION

Two principal methods for the control of expression are known. These are referred to in the art as "antisense downregulation" and "sense downregulation" or "cosuppression". Both of these methods lead to an inhibition of expression of the target gene. Overexpression is achieved by insertion of one or more than one extra copies of the selected gene. Other lesser used methods involve modification of the genetic control elements, the promoter and control sequences, to achieve greater or lesser expression of an inserted gene.

In antisense downregulation, a DNA which is complementary to all or part of the target gene is inserted into the genome in reverse orientation and without its translation initiation signal. The simplest theory is that such an antisense gene, which is transcribable but not translatable, produces mRNA which is complementary in sequence to mRNA product transcribed from the endogenous gene: that antisense mRNA then binds with the naturally produced "sense" mRNA to form a duplex which inhibits translation of the natural mRNA to protein. It is not necessary that the inserted antisense gene be equal in length to the endogenous gene sequence: a fragment is

sufficient. The size of the fragment does not appear to be particularly important. Fragments as small as 40 or so nucleotides have been reported to be effective. Generally somewhere in the region of 50 nucleotides is accepted as sufficient to obtain the inhibitory effect. However, it has to be said that fewer nucleotides may very well work: a greater number, up to the equivalent of full length, will certainly work. It is usual simply to use a fragment length for which there is a convenient restriction enzyme cleavage site somewhere downstream of fifty nucleotides. The fact that only a fragment of the gene is required means that not all of the gene need be sequenced. It also means that commonly a cDNA will suffice, obviating the need to isolate the full genomic sequence.

The antisense fragment does not have to be precisely the same as the endogenous complementary strand of the target gene. There simply has to be sufficient sequence similarity to achieve inhibition of the target gene. This is an important feature of antisense technology as it permits the use of a sequence which has been derived from one plant species to be effective in another and obviates the need to construct antisense vectors for each individual species of interest. Although sequences isolated from one species may be effective in another, it is not infrequent to find exceptions where the degree of sequence similarity between one species and the other is insufficient for the effect to be obtained. In such cases, it may be necessary to isolate the species-specific homologue.

Antisense downregulation technology is well-established in the art. It is the subject of several textbooks and many hundreds of journal publications. The principal patent reference is European Patent No. 240,208 in the name of Calgene Inc. There is no reason to doubt the operability of antisense technology. It is well-established, used routinely in laboratories around the world and products in which it is used are on the market.

Both overexpression and downregulation are achieved by "sense" technology. If a full length copy of the target gene is inserted into the genome then a range of phenotypes is obtained, some overexpressing the target gene, some underexpressing. A population of plants produced by this method may then be screened and individual phenotypes isolated. As with antisense, the inserted sequence is lacking in a translation initiation signal. Another similarity with antisense is that the inserted sequence need not be a full length copy. Indeed, it has been found that the distribution of over-and under-expressing phenotypes is skewed in favour of underexpression and this is advantageous when gene inhibition is the desired effect. For overexpression, it is preferable that the inserted copy gene retain its translation initiation codon. The principal patent reference on cosuppression is European Patent 465,572 in the name of DNA Plant Technology Inc. There is no reason to doubt the operability of sense/co-suppression technology. It is well established, used routinely in laboratories around the world and products in which it is used are on the market.

Sense and antisense gene regulation is reviewed by Bird and Ray in *Biotechnology and Genetic Engineering Reviews* 9: 207-227 (1991). The use of these techniques to control selected genes in tomato has been described by Gray et al., *Plant Molecular Biology*, 19: 69-87 (1992).

Gene control by any of the methods described requires insertion of the sense or antisense sequence, with appropriate promoters and termination sequences containing polyadenylation signals, into the genome of the target plant species by transformation, follow by regeneration of the transformants into whole plants. It is probably fair to say that transformation methods exist for most plant species or can be obtained by adaptation of available methods.

For dicotyledonous plants the most widely used method is *Agrobacterium*-mediated transformation. This is the best known, most widely studied and, therefore, best understood of all transformation methods. The rhizobacterium *Agrobacterium*

*tumefaciens*, or the related *Agrobacterium rhizogenes*, contain certain plasmids which, in nature, cause the formation of disease symptoms, crown gall or hairy root tumours, in plants which are infected by the bacterium. Part of the mechanism employed by *Agrobacterium* in pathogenesis is that a section of plasmid DNA which is bounded by right and left border regions is transferred stably into the genome of the infected plant. Therefore, if foreign DNA is inserted into the so-called "transfer" region (T-region) in substitution for the genes normally present therein, that foreign gene will be transferred into the plant genome. There are many hundreds of references in the journal literature, in textbooks and in patents and the methodology is well-established. Agrobacterium-mediated transformation of the cultivated strawberry (*Fragaria x ananassa* Duch.) is described in Plant Science, 69, 79-94 (1990).

The effectiveness of *Agrobacterium* is restricted to the host range of the micro-organism and is thus restricted more or less to dicotyledonous plant species. In general monocotyledonous species, which include the important cereal crops, are not amenable to transformation by the *Agrobacterium* method. Various methods for the direct insertion of DNA into the nucleus of monocotyledon cells are known.

In the ballistic method, microparticles of dense material, usually gold or tungsten, are fired at high velocity at the target cells where they penetrate the cells, opening an aperture in the cell wall through which DNA may enter. The DNA may be coated on to the microparticles or may be added to the culture medium.

In microinjection, the DNA is inserted by injection into individual cells via an ultrafine hollow needle.

Another method, applicable to both monocotyledons and dicotyledons, involves creating a suspension of the target cells in a liquid, adding microscopic needle-like material, such as silicon carbide or silicon nitride " whiskers", and agitating so that the cells and whiskers collide and DNA present in the liquid enters the cell.

In summary, then, the requirements for both sense and antisense technology are known and the methods by which the required sequences may be introduced are known. What remains, then is to identify genes whose regulation will be expected to have a desired effect, isolate them or isolate a fragment of sufficiently effective length, construct a chimeric gene in which the effective fragment is inserted between promoter and termination signals, and insert the construct into cells of the target plant species by transformation. Whole plants may then be regenerated from the transformed cells.

This invention is concerned with the control of ripening in fruit, and the particular interest here is in strawberries.

The interest in controlling the ripening process is to improve the flavour and/or texture of the fruit, both characters being largely affected by the ripening process. Sugars are the most important soluble component of the flavour. Some 99% of the soluble sugars in strawberry are accounted for by sucrose, glucose and fructose, the amount of these sugars being affected by the season but their relative proportions are largely unaffected.

There is little information in the literature on the metabolic pathways involved in the synthesis of sugars in strawberry. It is known, however that sugars are synthesised during the ripening of the fruit.

The changes in gene expression during strawberry fruit ripening and their regulation by auxin have been described in *Planta* 194: 62-68 (1994)

## OBJECT OF THE INVENTION

An object of the present invention is to provide DNA sequences enabling the construction of vectors suitable for genetic transformation of strawberry plants, with a view to control of the ripening process in strawberry fruit.

## SUMMARY OF THE INVENTION

According to the present invention there is provided a vector for use in the genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region, or a fragment of at least 10 bases thereof, of a strawberry protein selected from O-methyl transferase, acyl carrier protein (ACP), elongation factor, auxin-induced gene, cysteine(thiol) proteinase, cellulase, starch phosphorylase, pyruvate decarboxylase, chalcone reductase, protein kinase, auxin-related gene, sucrose transporter, meristem pattern gene, or selected from a strawberry protein with homology to transcribed sequence accession number T45086, transcribed sequence accession number L36159 or transcribed sequence accession number T45902, or selected from a strawberry protein of unknown homology encoded by one of the StrawRipe sequences A to K.

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The gene regulation sequence may be in the same or antisense orientation as the endogenous target gene. It may also be of partial or full sequence length. The invention further contemplates the overexpression of one or more of the genes by inserting into the strawberry genome one or more than one extra copy thereof.

The invention also provides a gene regulation sequence which comprises the coding region, or a fragment of at least 10 bases thereof, of a strawberry protein selected from O-methyl transferase, acyl carrier protein (ACP), elongation factor, auxin-induced gene, cysteine(thiol) proteinase, cellulase, starch phosphorylase, pyruvate decarboxylase, chalcone reductase, protein kinase, auxin-related gene, sucrose

transporter, meristem pattern gene, or selected from a strawberry protein with homology to transcribed sequence accession number T45086, transcribed sequence accession number L36159 or transcribed sequence accession number T45902, or selected from a strawberry protein of unknown homology encoded by one of the StrawRipe sequences A to K.

The sequences of this invention can also be used as probes for isolation of similar sequences from the strawberry genome.

The invention also provides a strawberry plant and propagating material thereof which contains a vector of this invention.

Further according to the invention, there is provided a method for altering the phenotype of strawberry plants, with the aim of controlling the ripening of strawberry fruit, comprising inserting into the genome of the cell of a strawberry plant a gene regulation vector of this invention.

In this way, the invention further provides genetically modified strawberry plants, propagation material and strawberry fruit.

## PREFERRED EMBODIMENTS

In the present invention, the regulation sequence comprises the coding region, or a fragment of at least 10 bases thereof, of a strawberry protein. The strawberry protein is selected from O-methyl transferase, acyl carrier protein (ACP), elongation factor, auxin-induced gene, cysteine(thiol) proteinase, cellulase, starch phosphorylase, pyruvate decarboxylase, chalcone reductase, protein kinase, auxin-related gene, sucrose transporter, meristem pattern gene, or selected from a strawberry protein with homology to transcribed sequence accession number T45086, transcribed sequence

accession number L36159 or transcribed sequence accession number T45902, or selected from a strawberry protein of unknown homology encoded by one of the StrawRipe sequences A to K.

Examples of suitable regulation sequences are SEQ ID NO:1 to SEQ ID NO:27, also referred to herein as Sequences 1 to 27. Related sequences taken from the priority documents of the present PCT application are given in SEQ ID NO:28 to SEQ ID NO:38.

The gene regulation sequences of the invention may be synthesised from the sequence information given or may be isolated from a library. To assist isolation Zeneca Limited have deposited with the National Collection of Industrial & Marine Bacteria, St. Machar Drive, Aberdeen, UK, a cDNA library of strawberry ripening genes. The library was deposited on 15th November 1994 under the Budapest Treaty and has the Accession Number NCIMB 40690.

Thus, this invention is based on the identification of genes which encode proteins implicated in strawberry ripening-related processes. DNA sequences which encode these proteins have been cloned and some have been characterised. The DNA sequences may be used to modify plants with the goal of modifying the ripening characteristics of fruit.

By virtue of this invention strawberry plants can be generated which, amongst other phenotypic modifications, may have one or more of the following fruit characteristics:

improved resistance to damage during harvest, packaging and transportation due to slowing of the ripening and over-ripening processes;

longer shelf life and better storage characteristics due to reduced activity of degradative pathways (e.g. cell wall hydrolysis),

improved processing characteristics due to changed activity of proteins/enzymes contributing to factors such as viscosity, solids, pH, elasticity;

improved flavour and aroma at the point of sale due to modification of the sugar/acid balance and other flavour and aroma components responsible for characteristics of the ripe fruit;

modified colour due to changes in activity of enzymes involved in the pathways of pigment biosynthesis (e.g. lycopene,  $\beta$ -carotene, chalcones and anthocyanins), increased resistance to post-harvest pathogens such as fungi.

The activity of the ripening-related proteins may be either increased or reduced depending on the characteristics desired for the modified plant part (fruit, leaf, flower, etc). The levels of protein may be increased; for example, by incorporation of additional genes. The additional genes may be designed to give either the same or different spatial and temporal patterns of expression in the fruit. "Antisense" or "partial sense" or other techniques may be used to reduce the expression of ripening-related protein.

The activity of each ripening-related protein or enzyme may be modified either individually or in combination with modification of the activity of one or more other ripening-related proteins/enzymes. In addition, the activities of the ripening-related proteins/enzymes may be modified in combination with modification of the activity of other enzymes involved in fruit ripening or related processes.

DNA constructs according to the invention may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA.

There is no theoretical upper limit to the base sequence - it may be as long as the relevant mRNA produced by the cell - but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length. The preparation of such constructs is described in more detail below.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used. The isolation of suitable ripening-related sequences is described above; it is convenient to use DNA sequences derived from the ripening-related clones deposited at NCIMB in Aberdeen. Sequences coding for the whole, or substantially the whole, of the appropriate ripening-related protein may thus be obtained. Suitable lengths of this DNA sequence may be cut out for use by means of restriction enzymes. When using genomic DNA as the source of a base sequence for transcription it is possible to use either intron or exon regions or a combination of both.

In a variation of the vector of this invention the regulation sequence varies from Sequences 1 to 27 but retains sufficient similarity to be effective in gene regulation. Thus, the regulatory gene may be a homologue of a gene of Sequence 1 to 27 which has been obtained from a strawberry plant.

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To obtain constructs suitable for expression of the appropriate ripening-related sequence in plant cells, the cDNA sequence as found in one of the strawberry plasmids or the gene sequence as found in the chromosome of the strawberry plant may be used. Recombinant DNA constructs may be made using standard techniques. For example, the DNA sequence for transcription may be obtained by treating a vector containing said sequence with restriction enzymes to cut out the appropriate segment. The DNA sequence for transcription may also be generated by annealing and ligating synthetic oligonucleotides or by using synthetic oligonucleotides in a polymerase chain reaction (PCR) to give suitable restriction sites at each end. The DNA sequence is then cloned into a vector containing upstream promoter and downstream terminator sequences. If

antisense DNA is required, the cloning is carried out so that the cut DNA sequence is inverted with respect to its orientation in the strand from which it was cut.

Promoters suitable for use in constructs of the invention may be any suitable promoters which are known to be effective in driving expression of foreign genes in plants, for example the promoters may be those which are isolatable from the genomic version of the cDNAs of the invention.

In a construct expressing antisense RNA, the strand that was formerly the template strand becomes the coding strand, and vice versa. The construct will thus encode RNA in a base sequence which is complementary to part or all of the sequence of the ripening-related RNA. Thus the two RNA strands are complementary not only in their base sequence but also in their orientations (5' to 3').

In a construct expressing sense RNA, the template and coding strands retain the assignments and orientations of the original plant gene. Constructs expressing sense RNA encode RNA with a base sequence which is homologous to part or all of the sequence of the mRNA. In constructs which express the functional ripening-related protein, the whole of the coding region of the gene is linked to transcriptional control sequences capable of expression in plants.

For example, constructs according to the present invention may be made as follows. A suitable vector containing the desired base sequence for transcription is treated with restriction enzymes to cut the sequence out. The DNA strand so obtained is cloned (if desired, in reverse orientation) into a second vector containing the desired promoter sequence and the desired terminator sequence. Suitable promoters include the 35S cauliflower mosaic virus promoter, the polyubiquitin promoter and the tomato polygalacturonase gene promoter sequence (Bird et al, 1988, Plant Molecular Biology, 11:651-662) or other developmentally regulated fruit promoters. Suitable terminator

sequences include that of the *Agrobacterium tumefaciens* nopaline synthase gene (the nos 3' end).

The transcriptional initiation region (or promoter) operative in plants may be a constitutive promoter (such as the 35S cauliflower mosaic virus promoter) or an inducible or developmentally regulated promoter (such as fruit-specific promoters), as circumstances require. For example, it may be desirable to modify ripening-related protein activity only during fruit development and/or ripening. Use of a constitutive promoter will tend to affect ripening-related protein levels and functions in all parts of the plant, while use of a tissue specific promoter allows more selective control of gene expression and affected functions. Thus in applying the invention it may be found convenient to use a promoter that will give expression during fruit development and/or ripening. Thus the antisense or sense RNA is produced only in the organ in which its action is required and/or only at the time required. Fruit development and/or ripening-specific promoters that could be used include the ripening-enhanced polygacturonase promoter (PCT/WO 92/08798), the E8 promoter (Diekman & Fischer, 1988, EMBO, 7:3315-3320), the fruit specific 2AII promoter (Pear et al, 1989, Plant Molecular Biology, 13:639-651), the histidine decarboxylase promoter (HDC, Sibia) and the phytoene synthase promoter.

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Ripening-related protein or enzyme activity (and hence ripening-related processes and fruit ripening characteristics) may be modified to a greater or lesser extent by controlling the degree of the appropriate ripening-related protein's sense or antisense mRNA production in the plant cells. This may be done by suitable choice of promoter sequences, or by selecting the number of copies or the site of integration of the DNA sequences that are introduced into the plant genome. For example, the DNA construct may include more than one DNA sequence encoding the ripening-related protein or more than one recombinant construct may be transformed into each plant cell.

The activity of each ripening-related protein may be separately modified by transformation with a suitable DNA construct comprising a ripening-related sequence. In addition, the activity of two or more ripening-related proteins may be simultaneously modified by transforming a cell with two or more separate constructs. Alternatively, a plant cell may be transformed with a single DNA construct comprising both a first ripening-related sequence and a second ripening-related sequence.

It is also possible to modify the activity of the ripening-related protein(s) while also modifying the activity of one or more other enzymes. The other enzymes may be involved in cell metabolism or in fruit development and ripening. Cell wall metabolising enzymes that may be modified in combination with a ripening-related protein include but are not limited to: pectin esterase, polygalacturonase,  $\beta$ -galactanase,  $\beta$ -glucanase. Other enzymes involved in fruit development and ripening that may be modified in combination with a ripening-related protein include but are not limited to: ethylene biosynthetic enzymes, carotenoid biosynthetic enzymes including phytoene synthase, carbohydrate metabolism enzymes including invertase.

Several methods are available for modification of the activity of the ripening-related protein(s) in combination with other enzymes. For example, a first plant may be individually transformed with a ripening-related gene construct and then crossed with a second plant which has been individually transformed with a construct encoding another enzyme. As a further example, plants may be either consecutively or co-transformed with ripening-related constructs and with appropriate constructs for modification of the activity of the other enzyme(s). An alternative example is plant transformation with a ripening-related construct which itself contains an additional gene for modification of the activity of the other enzyme(s). The ripening-related gene constructs may contain sequences of DNA for regulation of the expression of the other enzyme(s) located adjacent to the ripening-related sequences. These additional sequences may be in either sense or antisense orientation as described in PCT/WO 93/23551 (single construct having distinct DNA regions homologous to different target

genes). By using such methods, the benefits of modifying the activity of the ripening-related proteins may be combined with the benefits of modifying the activity of other enzymes.

A DNA construct of the invention is transformed into a target plant cell. The target plant cell may be part of a whole plant or may be an isolated cell or part of a tissue which may be regenerated into a whole plant. For any particular plant cell, the ripening-related sequence used in the transformation construct may be derived from the same plant species, or may be derived from any other plant species (as there will be sufficient sequence similarity to allow modification of related isoenzyme gene expression).

Transgenic plants and their progeny may be used in standard breeding programmes, resulting in improved plant lines having the desired characteristics. For example, fruit-bearing plants expressing a ripening-related construct according to the invention may be incorporated into a breeding programme to alter fruit-ripening characteristics and/or fruit quality. Such altered fruit may be easily derived from elite lines which already possess a range of advantageous traits after a substantial breeding programme: these elite lines may be further improved by modifying the expression of a single targeted ripening-related protein/enzyme to give the fruit a specific desired property.

By transforming plants with DNA constructs according to the invention, it is possible to produce plants having an altered (increased or reduced) level of expression of one or more ripening-related proteins, resulting from the presence in the plant genome of DNA capable of generating sense or antisense RNA homologous or complementary to the RNA that generates such ripening-related proteins. For fruit-bearing plants, fruit may be obtained by growing and cropping using conventional methods. Seeds may be obtained from such fruit by conventional methods (for example, tomato seeds are separated from the pulp of the ripe fruit and dried, following

which they may be stored for one or more seasons). Fertile seed derived from the genetically modified fruit may be grown to produce further similar modified plants and fruit.

The fruit derived from genetically modified plants and their progeny may be sold for immediate consumption, raw or cooked, or processed by canning or conversion to soup, sauce or paste. Equally, they may be used to provide seeds according to the invention.

The genetically modified plants (transformed plants and their progeny) may be heterozygous for the ripening-related DNA constructs. The seeds obtained from self fertilisation of such plants are a population in which the DNA constructs behave like single Mendelian genes and are distributed according to Mendelian principles: e.g., where such a plant contains only one copy of the construct, 25% of the seeds contain two copies of the construct, 50% contain one copy and 25% contain no copy at all. Thus not all the offspring of selfed plants produce fruit and seeds according to the present invention, and those which do may themselves be either heterozygous or homozygous for the defining trait. It is convenient to maintain a stock of seed which is homozygous for the ripening-related DNA construct. All crosses of such seed stock will contain at least one copy of the construct, and self-fertilized progeny will contain two copies, i.e. be homozygous in respect of the character. Such homozygous seed stock may be conventionally used as one parent in F1 crosses to produce heterozygous seed for marketing. Such seed, and fruit derived from it, form further aspects of our invention. We further provide a method of producing F1 hybrid plants expressing a ripening-related DNA sequence which comprises crossing two parent lines, at least one of which is homozygous for a ripening-related DNA construct. A process of producing F1 hybrid seed comprises producing a plant capable of bearing genetically modified fruit homozygous for a ripening-related DNA construct, crossing such a plant with a second homozygous variety, and recovering F1 hybrid seed. It is possible according to our invention to transform two or more plants with different ripening-related DNA

constructs and to cross the progeny of the resulting lines, so as to obtain seed of plants which contain two or more constructs leading to reduced expression of two or more fruit-ripening-related proteins.

## EXAMPLES OF THE INVENTION

The invention will now be described, by way of illustration, by the following Examples. In the Examples, reference is made to Figure 1.

## THE DRAWING

Figure 1 is a diagrammatic map of plasmid pBINCEL.

## EXAMPLE 1

### Construction of a cDNA library of ripening genes

#### 1.1 Isolation of messenger RNA

Total RNA was isolated from ripe fruit tissue (the receptacle with the achenes removed) of strawberry (*Fragaria x ananassa* Duch. cv. Brighton) as described by Manning K. *Analytical Biochemistry* 195, 45-50 (1991). Messenger RNA was isolated from total RNA by oligo(dT)-cellulose chromatography according to Bantle et al., *Analytical Biochemistry* 72, 413-427 (1976).

#### 1.2 Synthesis of cDNA

The first and second strands of the cDNAs were synthesised from messenger RNAs using a commercial cDNA synthesis kit (RPN 1256Y: Amersham Life Sciences, Amersham, Bucks., UK), priming the first strand cDNA synthesis with oligo-dT.

### 1.3 Cloning into vector

Double stranded cDNAs were cloned into the λgt10 vector using the BRL cloning system (8287SA: Bethesda Research Laboratories, Paisley, Renfrewshire, UK) essentially as follows. Internal EcoRI sites of the cDNAs were methylated using EcoRI methylase. The DNA termini were repaired with T4 DNA polymerase and phosphorylated EcoRI linkers ligated to the cDNA with T4 ligase. Excess linkers were digested and removed by column chromatography on DEAE-Sephadex. The purified double stranded cDNAs with EcoRI termini were ligated into λgt10 vector DNA digested with EcoRI and dephosphorylated. Vector DNA was then packaged using an *in vitro* packaging extract (Promega Corporation, Southampton, UK). Recombinant bacteriophage were mixed with plating bacteria (*E. coli* C600 hflA 150) as described in the BRL protocol to determine titre, for library screening and subsequent amplification.

### 1.4 Screening of the cDNA library from ripe strawberry

The unamplified cDNA library from ripe strawberry was differentially screened using cDNA from fruit receptacle tissue at the ripe and white stages of ripeness. A proportion of the library was plated at low density and duplicate plaque lifts made on to Hybond N nylon filters (Amersham) according to the manufacturer's instructions. One filter was hybridised to ripe cDNA from white fruit and the duplicate filter hybridised to ripe cDNA. Hybridisations were at high stringency using digoxigenin as a non-radioactive label (Boehringer Mannheim, Lewes, Sussex, UK). Plaques hybridising preferentially to ripe cDNA were picked and replated at low density for a second round of selection by differential screening. Single plaques from the second screening were picked and numbered as ripening-enhanced clones.

### 1.5 Characterisation of the ripe cDNA library and ripening-enhanced clones

The ripe cDNA library was prepared with an efficiency of  $3.03 \times 10^6$  plaque-forming units per microgram of cDNA. The size of the cDNA inserts in this library ranged from approximately 0.24 to 6 kbp with a mean insert size of approximately 1.4 kbp.

From the 1343 plaques used in the first screen, 83 putative ripening clones were obtained. Of these, 48 were pure clones with single inserts, the remainder being impure and having multiple inserts.

The 48 clones with single inserts were partially sequenced using the DyeDeoxy (Trade Mark) Terminator Cycle Sequencing Kit (Applied Biosystems, Warrington, Cheshire, UK) with forward and reverse primers specific for the  $\lambda$ gt10 vector. Improved sequence data were obtained for clones with multiple inserts and clones with single inserts that did not produce good sequence data by subcloning into the phagemid vector pBK-CMV (Stratagene) vector for sequencing. From the sequenced clones, the following twenty-seven ripening-related clones were selected. Comparison of these sequences with sequences in the EMBL database using GCG ("Winconsin") software has identified homologies for the clones of sequences 1 to 16 listed in the following table 1.

Sequence ID NO	Homology/Identity	Clone number
1	O-methyl transferase	1
2	acyl carrier protein (ACP)	3
3	elongation factor	33a
4	auxin-induced gene	33b
5	cysteine(thiol) proteinase	93c
6	cellulase	97
7	starch phosphorylase	6ab

8	pyruvate decarboxylase	16bc
9	chalcone reductase	31c
10	protein kinase	75b
11	auxin-related gene	61c
12	sucrose transporter	110ab
13	meristem pattern gene	26
14	transcribed sequence, T45086	13
15	transcribed sequence, L36159	56
16	transcribed sequence, T45902	61b
17	StrawRipe A	10
18	StrawRipe B	40
19	StrawRipe C	48
20	StrawRipe D	54
21	StrawRipe E	62
22	StrawRipe F	81
23	StrawRipe G	90
24	StrawRipe H	92
25	StrawRipe I	99
26	StrawRipe J	106b
27	StrawRipe K	106c

### 1.6 Expression of ripening enhanced clones

RNA was extracted from strawberry fruit during normal development and analysed by Northern blotting using standard procedures. The level of messenger RNA corresponding to the expression of O-methyl transferase, cysteine proteinase, acyl carrier protein and auxin induced gene were monitored in the receptacle at various time points between pollination and the overripe stage, between Day 1 and Day 19, and then at the stages of Turning, Orange, Ripe and Overripe. Messenger RNA for O-methyl transferase appeared at Day 19,

through to Overripe and was highest at Orange and Ripe. The messenger RNA for cysteine proteinase was low up to day 19, and then increased between the Turning and Overripe stages. The messenger RNA for Acyl carrier protein was low up to Day 19, and increased for Turning, Orange and Ripe. The messenger RNA for Auxin induced gene appeared around Day 16, and was highest between the Turning and Overripe Stages.

The data provide evidence that O-methyl transferase, cysteine proteinase, acyl carrier protein and auxin induced gene are involved in the ripening process in normal fruit development.

## EXAMPLE 2

### Construction of antisense RNA vectors with the CaMV35S promoter

A vector is constructed using the sequences corresponding to a fragment of one of the sequences 1 to 38, more especially one of the sequences 1 to 27. This fragment is synthesised by the polymerase chain reaction using synthetic primers. The ends of the fragment are made flush with T4 polymerase and it is cloned into a derivative of the pBINPLUS vector (van Engelen *et al.*, Transgenic Research 4, 288-290 (1995)) containing the cauliflower mosaic virus (CaMV) 35S promoter-nopaline synthase (nos) 3' terminator cassette inserted into the HindIII/EcoRI site. For example, in this way, the plasmid pBINCEL is obtained which is derived from pBINPLUS and which contains cellulase cDNA in either the sense or antisense orientation. A diagrammatic map of the plasmid pBINCEL is given in Figure 1. In one particular experiment, an antisense extended sequence comprising the cellulase of SEQ ID:6: with the addition of a polyA tail of 17 bases was inserted to give a pBINCEL antisense cellulase vector.

Alternatively a vector is constructed using a restriction fragment obtained from a strawberry ripening-related clone. The fragment is blunt ended with T4 polymerase and is cloned into a derivative of the pBINPLUS vector.

After synthesis of the vector, the structure and orientation of the sequences are confirmed by DNA sequence analysis.

#### EXAMPLE 3

##### Construction of antisense RNA vectors with a fruit enhanced promoter.

The fragment of the ripening-related cDNA that was described in Example 2 is also cloned into the vector pJR3. pJR3 is a Bin 19 based vector, which permits the expression of the antisense RNA under the control of the tomato polygalacturonase (PG) promoter. This vector includes approximately 5 kb of promoter sequence and 1.8 kb of 3' sequence from the PG promoter separated by a multiple cloning site

After synthesis, vectors with the correct orientation of the ripening-related sequences are identified by DNA sequence analysis.

Alternative fruit enhanced promoters (E8, 2A11 or any strawberry promoter) are substituted for the polygalacturonase promoter in pJR3 or for the CaMV 35S promoter in the modified pBINPLUS vector described in Example 2 to give alternative patterns of expression.

#### EXAMPLE 4

##### Construction of truncated sense RNA vectors with the CaMV 35S promoter

The fragment of the ripening-related cDNA that was described in Example 2 is also cloned into the vectors described in Example 2 in the sense orientation.

After synthesis, the vectors with the sense orientation of the phytoene synthase sequence are identified by DNA sequence analysis.

#### EXAMPLE 5

Construction of truncated sense RNA vectors with fruit-enhanced promoter.

The fragment of the ripening-related cDNA that was described in Example 3 is, also cloned into the vectors described in Example 3 in the sense orientation.

After synthesis, the vectors with the sense orientation of the ripening-related sequence are identified by DNA sequence analysis.

#### EXAMPLE 6

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Construction of an over-expression vector using the CaMV35S promoter

The complete sequence of a ripening-related cDNA containing a full open-reading frame is inserted into the vectors described in Example 2.

#### EXAMPLE 7

Construction of an over-expression vector using a fruit-enhanced promoter

The complete sequence of a ripening-related cDNA containing a full open-reading frame is inserted into the vectors described in Example 3 (pJRG or alternatives with different promoters).

#### EXAMPLE 8

##### Generation of transformed plants

Vectors are transferred to *Agrobacterium tumefaciens* EHA105 (a kanamycin sensitive strain of an organism widely available to plant biotechnologists; Hood et al., Transgenic Research 2, 208-218 (1990)) and are used to transform strawberry plants. Strawberry explants infected with *Agrobacterium* are grown on regeneration medium normally containing 100 mg/l kanamycin. After three weeks, the explants are transferred to regeneration medium without kanamycin. At 4 to 6 weeks, putatively transformed shoots are cultured on propagation medium for two weeks and then transformants are selected on medium containing 25 mg/l kanamycin. Regenerated plants containing the transgene are selected and grown to maturity. Ripening fruit are analyzed for modifications to their ripening characteristics.

For example, transformed plants were produced in this way using the pBINCEL antisense cellulase fragment of Example 2. The presence of the transgene in the putative strawberry transformant was verified by PCR using genomic DNA from the transformant as template and primers from the 35S promoter and from the cellulase strand. The PCR products were separated by agarose gel electrophoresis and a fragment of ~1400 base pairs was obtained that was identical in size to the PCR product obtained using the pBINCEL antisense cellulase vector DNA as template.

The following sequences have been edited to remove vector bases and polyA regions, as appropriate.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

## (i) APPLICANT

(A) NAME: Horticulture Research International  
(B) STREET:  
(C) CITY: Stratford-upon-Avon  
(D) STATE OR PROVINCE: Warwick  
(E) COUNTRY: United Kingdom  
(F) POSTAL CODE: CV35 9EF

(ii) TITLE OF INVENTION: Fruit Ripening-Related Genes

(iii) NUMBER OF SEQUENCES: 38

## (iv) COMPUTER-READABLE FORM:

(A) MEDIUM TYPE: 1.44 MB Diskette  
(B) COMPUTER: DELL Pentium  
(C) OPERATING SYSTEM: Windows  
(D) SOFTWARE: Word

## (2) INFORMATION FOR SEQ ID NO. 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 549  
(B) TYPE: cDNA  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: O-methyl transferase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:1:

CCNCCNNCTC	AATNTNNNNC	ATCATNTNTN	NGGGGGTTGG	GGNTCTNGAA	050
GGCAAAAGAT	TCGGTCAGGA	CAAGGTCTC	GTCGAGAGCT	GGTATCATT	100
GANGGATGCA	GTTCTTGATG	GTGGGATTCC	ATTTAACAAAG	GNCTATGGCA	150
TGACTGCATT	TGATTACCAT	GGNAACTGAC	CCTAGCATT	AACAAGGTCT	200
TCAACAAGGG	AATGGCTGAC	CACTCCACCA	TTACCATGCA	NGTAAAATCC	250
TTGTAGTACT	TACAAAGGCT	TCGAGGGCCT	CAAATCCATC	GTTGTATGTC	300
GGTGGCGGNA	CCNGAGCTGT	GGNGAACAT	NATCGCTTCC	CNAGTTNCCC	350
TTCGCATCAA	GGGTCACTCAN	CCTTTCGACT	TGCCCTCAAT	CTTANTCGAA	400
NGCATTCTC	CNTCAATTAT	CCTNNNTGTT	TCCANCCANG	TTGGGATGNG	450
GGGANAAATCT	TCTGGCNANN	TCTTACCCAA	TTNNNGNANN	CTTCCATTCT	500
TTCCCATTTN	AGTCNTNTT	TTNCTCAACC	TAACTTGNCG	NTCCNTCGN	549

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	661
(B) TYPE:	cDNA
(C) STRANDEDNESS:	Single
(D) TOPOLOGY:	Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Acyl carrier protein

(xi) SEQUENCE DESCRIPTION: SEQ ID:2:

GGTTTTAGAA	CTATCCTCGA	TCGCATCAAT	GGCCGCCACC	ACAGGAGCTG	050
CTTCTTCGAT	CTCACTCCGC	TCTCGCCTTC	ACCAGAACATCT	TGCATCGTCC	100
AGGGTCAATG	GTCTTAAGCC	AGTTTTACTG	TCTGGTAATG	GAAGAAGTTC	150
TCTTTCTTTC	GGGTTACAGA	AGCGTTCAGC	ACGGCTTCAG	ATTACTGCG	200
CAGCCAAACC	AGAGACAATG	GACAAGGTGT	GCCAGATAGT	TAGAAAGCAA	250
CTTGCATTAC	CAGATGACTC	GGCAGTTCT	GGAGAGTCAA	AATTTCTGC	300
ACTTGGAGCT	GATTCTCTTG	ATACGGTTGA	GATCGTGATG	GGACTTGAGG	350
AGGAATTTGG	TTTTAGCGTG	GAAGAGGAGA	GTGCTCAGAG	CATTGCAACC	400
GTCAGGATG	CTGCGGATCT	TATCGAGAAG	CTCATTGAGA	AGAACAAATGC	450
TTAGAAGAAG	AAATGAGAAA	ACAAGAGTCA	ATCCTAGCCT	GCTTTAGATA	500
ATTATTTGGT	TGGTAGACTG	GTTATGTATG	CAGTCATTTT	GTGTGAAATT	550
TGAACCTGAT	AGTGGCTTGA	GTGTTAAATT	ATGAATGTAT	GGATTGAGT	600
TTGTGTGGTC	AAGCTCCTTT	CTTCCTATA	TTTCTGATGA	AATAGAGAAT	650
GGCCTTACAA	T				661

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1026  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Linear  
 (D) TOPOLOGY: Single

## (ix) FEATURES

(D) OTHER INFORMATION: Elongation factor

## (xi) SEQUENCE DESCRIPTION: SEQ ID:3:

GGGCCATGT	TGACAAAGCT	CAATGTCACT	ATGAAGAGTG	ATGAAAAAGA	050
ACTTATGGGA	AAGGCATTGA	TGAAGAGGGT	CATGCAGAAC	TGGCTTCAG	100

CCAGCACTGC CCTATTGGAA ATGATGATCT TTCACCTTCC CTCTCCACAC	150
ACAGCTAAA AGTACCGTGT TGAGAATTG TACGAGGGTC CCCTGGATGA	200
CCAATATGCT AATGCTATCA GAAACTGTGA TCCAGATGGT CCGCTTATGC	250
TTGTATTGTA TCTAAGATGA TTCCGGCATC TTGACAAGGG TNAGATTCTT	300
TGGTTTGAGG TCGTGTGTT TGGCTGGTAG GGGTCCAAA CTGGTTGGA	350
NGGGTTAAGG AATTATGGGG ACCCAAAC TA TTGTTCTGG GGAAAAGAGG	400
GATCTTATG TCAAGAATTG TACAGNGGA CTTGNNACT TGGATGGGA	450
AAAGAAACAA NGAAACTGTT GAGGATGTT CCCTGTGGTA AAAACTTGTN	500
CCCTTGGTTG GTCTGGGAAN AAGTTCAATC CACCCAAAGAA TGCTACCTTG	550
ACCAAATGAG AGGGNAACAA GATGCTCCCC CCATTCTGTC AATGAAGTT	600
TCCTGTCTCA ACCCTGTTGT GCGTGTGCT GTTCAANC GT AAGGNTGCTT	650
CTTGATCCCTT CCCCCAAGCTT GTTGAAGGGC TGAAACGTCT GGCTAAGACC	700
CGATCCCTAT GGGTGTCTGT ACCATTGAGG AGTCTGGAGA GCACATCATT	750
GCTGGAGCTG GTGAACCTCA CCTTGAGATC TNCNTGANGG ATCTINCAAGA	800
TGATTTTATG GGTGGAGCGG AAATTGTAAA ATCTGATCCT GTTGTGTCCT	850
NCCGTGAGAC AGTCCTTGAG AAGNCCTNCC GTACTGTGAT GAGCAAGTCT	900
CCCAACAAGC ACAACCGTCT GTACATGGAA GCACNCCGT TGGAGGAAGG	950
TCTTCCTGAG NCCATTGATG ATGGTCGTAT TGGNCCAAGG GATGATCCTA	1000
AAATCCGCTC AAAGATCTTG NCTGAG	1026

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 957

(B) TYPE: cDNA

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Auxin-induced mRNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID:4:

GGCACCAAGTG	CTTCATATCT	CGCCCTTG	AGTTTCACAT	ATCAAAGTAG	050
CATCTCAAAT	CACATCAATG	GCAGACGAGG	TTGTCTTGT	GGACTCTGG	100
CCAAGCCC	TTGGGATGAG	GCTGAGGATC	GCTCTGCCG	AGAAAGGC	150
CAAGTACGAG	TACAAGGACG	AGGACCTGAG	GAACAAGAGC	CCGCTGTTGC	200
TTCAAGTCAA	CCCGGTTCAC	AAGAAGGATC	CCGGTTCTCA	TTCACAAACGG	250
CAAACGTCT	TGCGAGTCTT	GTCATTGCTC	TTCAAGTACA	TTGACCGAGG	300
CTTGGACTTA	ACAAAGCCAC	TATTGNCC	CCGACCC	ACCTCAGGAT	350
CCCCAGGCCA	GGGTCTTGGG	CCGACTTCCG	NGGACAAAGA	AGATNTTTTG	400
ATNTCGGGTA	GGNAAGACAA	TGGNCAACGA	AAGGAGATTG	AGCAGGGAGG	450
CAGNAAAGAA	GGGATTCTTC	GACTGCATTA	AGTTGCTAGA	AGTGGAGCTT	500
GGTGACAAGC	CTTTCTTGG	CGGTGAGACC	CTCGGATTG	TGGACGTGAC	550
GCTCGNTCCT	TTCTATTCT	GGTTCTCTGT	GTATGAGAAA	TACGGCAACT	600
TCAGCATTGC	GCCAGAGTGC	CCAAAGTNCA	TGGCTTGGGT	TAAGAGGTGT	650
ATGGAGAAGG	AGAGTGTGTC	AAAGTCTCTT	CCTGACCAGG	ACAAGGTCTG	700
TGGCTTNGTT	GCCGAGATGA	NGAAGAAGCT	TGGAGTTGAG	TAGATGTGAT	750
CAATGTCATN	TTGATCATGT	CTTTGTTTA	GCCCCAAGAT	TCANCCTCGT	800
TTTGGGTTGC	TTGTATTTT	CAATAAAATT	GGGGGACTTG	GACCAAGCCC	850
TCCAATAGTA	GGAAGCACTC	TTTCNGTGCC	TCTTGGTCCN	GTTCCTTCTTC	900
NGNTAACCT	NTNTGCAGCT	AAAATTCA	GNATTNCTGN	TTTCCTTNTA	950
TNGCCAA					957

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 518  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Cysteine (thiol) proteinase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:5:

ATCTCCTCCT	CCTCTCTCTC	CTTCTCCTCC	TCTCCTCCGC	CGTCGCCCTCC	050
ACCGTAACCG	ACGCCGGCGA	TCCTCTCAT	CGACAAGTCG	TACCGGGCGC	100
GGCCGAGGAT	GACCGAGCTCC	TCCACCGCGA	GCGTCACITC	TCGAACTTCA	150
AAGCCACGTT	CGGAAAGAGC	TACGCGAGCC	AGGAGGGAGCA	CGACTACAGG	200
TTCCGGCGTA	TTCAAGGNCA	ACTCCGCCGG	GCGAAGAGGC	ACCAGGGGCT	250
TGGACCCCAC	CGCCGTGCAC	GGTGTCAACG	AAATCTCCGA	TCTCACTCCC	300
AAGGAGTTTC	GNCGGAAATT	TCCTCGGGCT	TAAGAAGGGG	TCGGANTTCG	350
GGTTACCGGC	CGACGGTTAA	AAAAGGGGCC	NGATNCCTNC	CGGANGAATT	400
ANCTTCCCCA	CCCANTTTTG	GNNTTGGGGN	GAAAAAAGGN	GCCCGNCNAA	450
GNCGGNGGAA	NGGNCAAGGG	GGAAATNGGG	TNNAATTNGG	NCNGGTTNAN	500
NGNGGGCCCG	NAGAANTT				518

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1766  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Cellulase (endo-(1,4)beta-n-glucanase)

## (xi) SEQUENCE DESCRIPTION: SEQ ID:6:

GGCAGCAAAA ACGAGAGAGA AAAAAAAATG GCGCGAAATG GCCTTTGCTT 050  
 ACCGGGAAAT GCTCCCGCAT TTTCGCGAAC ACTCGTCCTC TCGCTGCTCC 100  
 TGCTTCTCCA GCCAATCCGC GCCGGCCACG ACTACCACGA CGCCCTCCGC 150  
 AAGAGCATCC TCTTCTTCGA AGGCCAGCGC TCCGGCAAGC TCCCGCCCGA 200  
 TCAACGCCTC AAATGGCGCC GCGACTCCGC ATTGCACGAC GGCTCCACCG 250  
 CCGCGTAGA CTTAACCGGC GGCTACTACG ACGCCGGCGA CAACGTGAAG 300  
 TTCGGGTTTC CGATGGCGTT CACGACCACT CTGCTGGCGT GGAGCATTAT 350  
 AGACTTCGGG AGGGTCATGG GGACGGAGCA GAGGAACGCG GTCAAGGCCT 400  
 TACGGTGGGG GACAGACTAC CTCCTGAAGG CCACGGCGGT TCCTGGCGTC 450  
 GTCTTCGTCC AAGTCGGCGA CCCATACTCC GATCACAACT GCTGGGAGAA 500  
 GCCGGAAGAC ATGGACACAC GCCGCACGGT GTACAAAATC GACCACAACA 550  
 ACCCGGGATC CGACGTGGCA GGCAGAACCG CAGCCGCGCT CGCCGCCGCC 600  
 TCCATCGTT TCAGGTACG TGACCCCGCT TACTCGAGAC TGCTTCTCAA 650  
 TCGAGCCGTT AAGGTTTCG AGTTCGCTGA TACCCACCGC GGCGCGTACA 700  
 GCTCCAGCCT CAAAAACGCC GTGTGCCCTT TTTACTGCGA CGTCAACGGC 750  
 TTCCAGGATG AGTTACTGTG GGGAGCAGCG TGGTTGCACA AGGCGTCGAG 800  
 AAGGCGGCAG TACAGAGAAT ACATAGTGAG AACAGAGGTC ATTTTGAGAG 850  
 CTGGAGATAC CATTAACGAG TTTGGTTGGG ATAACAAGCA TGCTGGGATT 900  
 AATATTCTCA TTTCTAAGGA AGTGTATATG GGAAAAGCAG ATTATTCGA 950  
 ATCTTCAG CAAAATGCAG ATGGATTAT ATGCTCTGTT TTGCCTGGAC 1000  
 TTGCCCATAC CCAAGTCCAA TATTCTCCAG GTGGTTTGAT CTTCAAGCCT 1050  
 GGAGGGAGTA ACATGCAGCA TGTAACCTCG CTATCGTTCC TGCTTTGAC 1100  
 TTATTCCAAC TATCTAAGCC ACGCCAATAA GAACGTGCCG TGTGGCATGA 1150  
 CCTCCGCCTC CCCGGCCTTC CTCAAACAAT TGGCTAAACG CCAGGTGGAT 1200  
 TACATTTGG GTGACAATCC ATTAAGAATG TCTTACATGG TTGGATATGG 1250  
 GCCCGCGTTAC CCGCAAANGA TTCACCAACCG GGGCAGCTCA CTTCCATCCG 1300

TGCAGGCCCA TCCGGCCCGT ATCGGATGCA AAGCCGGTTC TCATTATTTT 1350  
 CTGAGTCCGA ATCCAAACCC GAATAAAATTA GTCGGGGCTG TTGTGGGCGG 1400  
 ACCCAATAGC TCGGATGCAT TTCCGGACTC GAGGCCTTAC TTTCAAGAGT 1450  
 CTGAGCCCAC GACGTACATA AATGCGCCTC TTGTGGGCCT ACTTTCGTAT 1500  
 TTTGCAGCCC ATTACTAATT CTCGAAGTGT AAACAGTGAT TGAGAATTG 1550  
 TTGTGGTGCG CCAATACTCA CCCACCAATC CCCCCACACTA CCAATTGTTG 1600  
 TTACTTTGG AAAGTTCTAA ATTTAAGAAA TTGTTAAGAA AGAAAATGGC 1650  
 CCAAGCTTAG TTATGGAATT TAGTCTCAAA AGCCCTACTG TTGTGCTTTT 1700  
 GAAATGTTCT AGCTGTAACA TAATTTCTAT CAATGAATAA AGAAAATGGG 1750  
 CCAAGCCTAA ATGTGG 1766

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 585
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Starch phosphorylase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:7:

AATCCTGGGG GGNTTNCCCA CCCTTAANTT GGCGNNNGAT NTTTTTGATA 50  
 CTCNTCGGGG GGGCGGAANC CTATGGGGAG AANNGGCAAC CAAAGGNGCC 100  
 TTTTNTAGGG TTGCCTGGCN TATTTACTGG CCTGGTNCTN AACATGTNCT 150  
 TTCCTGCGAT ATCCCCTGAT TCTGNGGATA ANCCGTATNA CNCGCCNNTG 200  
 AGTGAGGCTG ATACCGCTNC ACCGCATCCG ACCGACCGAT CGCAGCGAGT 250  
 CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAGGCC ACCTCTCNCC 300

GCGCGTTGGC CGATTCA TTGAGCTGG CACGACAGGT TTCCCGACTG	350
GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CTCACTCATT	400
AGNCACCCCCA GGCTTACAC TTTATGCTTC CGGCTCGTAT GTTGTGTGGA	450
ATTGTGAGCG GATAACAATT TCACACAGGA AACANCTATG ACCATGATNA	500
CNCCAAGCTA TTTAGCTGAC ACTANAGCAT ACTCAAGCTT GNATGCCTAC	550
AGNTCGACTC TAGAGGATCC ACCGGGTACC GAGCT	585

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 693
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Pyruvate decarboxylase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:8:

CATCITTTCA CTCGAAGTCT CAATCTTCA TCACAAACAT TCCCATTGAA	050
TCACAAAAAA GTTCAACCT TTAAACCTCC ATGGACACCA AGATTGGCTC	100
CATCGACGTC TGCAAAACCG AGAACACACGA CGTCGGTTGT TTACCAAACA	150
GCGCCACCTC CACCGTTCAA AACTCAGTCC CTTCGACCTC CCTCAGCTCC	200
GCCGACGCCA CCCTCGGCCG CCACCTGGCA CGCCGCCTCG TTCAAATCGG	250
CGTCACCGAC GTCTCACCG TCCCCGGCGA CTTCAACTTG ACCCTTCTCG	300
ACCAACCTCAT CGCCGAGCCC GGCCTCACCA ACATTGGCTG CTGCAACGAG	350
CTCAACGCCG GGTACGCCGC CGACGGCTAC GCGCGGTGCG GTGGCGTCGG	400
CGCCGTTGCG TGTTGACTTT CACTGTTGGT GGACTGAGTG TGCTGAACGC	450
GATCGCCGGC GCGTTATAGT GAGAATTGCG CGGTGATTG TATTGTTGGT	500

GGGCCCAAC TTCTAATGAT TATGGACTA ACCGGATTCT TCACCATACT	550
ATTGGGTTGC CGGACTTCAN TTCAAGAACT CCGGTGGTT CAAGAACNTG	600
ACTTGCTTTT CAGGCTGTGG GTGAATAATT CTTGGAAGAA TGACATGAA	650
TTTGCTTGAA TACNGCAATT TTCAATNGCN TTNGAAANAA AAC	693

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 693  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Chalcone reductase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:9:

CCCAAATCCC AGAAGTGGTT CTTGAATCCT CCAACGGCCG CAGAACCATG	050
CCTGTGCTTG GATTGGCAC AGCATCCAAC AATTACAAAC CGGAGGTTTT	100
<hr/>	
GATAGAAGCT GTTCTTGAGG CCATCAAGCT TGGTTACCGA CACTTCGACA	150
CTGCTTCCAT TTACGGCTCC GAGCAGACTC TAGGAGTAGC CATTGCCAA	200
GCGCTCAAAC TCGGCCTCGT GGCTTCTCGT GACGAGCTCT TCATCACTTC	250
CAAGCTTGG CCTAATGATG GTCAACCCAA CCTGGTTATT CCTGCTCTCA	300
AGAAAATCGC TTCAGAATCT TGAGTTGGAG TACCTTGATT TGTATCTGAT	350
ACACTGGCCC ATCAGTGCCA AGCCTGGAA AGTTGAGTCA CGCACTAGAG	400
GGAGAAGGAC CAAATGCCGA TGGACTCAA GGGTGTGTGG GCAGACATGG	450
AGGAAGCTCA GAGACTTGGC CTCACCAAAT CCATTGGAA TCAGCAATT	500
CTCTACAAA AAGACTCAGA ATTGCTCTC CTTGGCTAC TATTCCCTCG	550
TCAGTCAATC AANTTAANA TGANTCCATT TTGGAACAG AAGAACCTCA	600

AAAACTTCTG CAAGGCCAGT GGTATAATT GTGACTGGCT TCTCCCCATT	650
GGGTGCCATN NGAACCANTT GGGGGCACCA ATCATGTTCT CNA	693

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 763
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Protein kinase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:10:

GCANANGTG TTGTGGAAC TGGTCATTT GGAATTGTAT TCCANGCGAA	050
ATGCTTGGAA ACTGGTGAGA CTGTGGCCAT AAAGAAGGTT TTACAGGACA	100
GAAGGTATAA GAACAGGGAA CTTCAATTGA TGCGCGTAAT GGATCATCCA	150
AATGTGATTG GTTTGAAGCA TTGTTTCITC TCTACAACAA GCAAAAATGA	200
GCTTTTCTC AATTGGTTA TGGAATATGT TCCGGAAACT ATGTATCGGG	250
TTATAAAAGCA TTACAGCAAT GCAAACCAGA AAATGCCCT TGTCTATGTC	300
AAACTTTACA TGTNCCACAT TTTCAGAGGG CTGGCTTACA TACACACCGT	350
TCCTGGAGTT TGCCATANAN ATTTGAANCC TCCAAATTAA TTGGTTGATC	400
CTCTTATTCA CCANGTCAAG CTTTGTGAT TTTGGAAGTG CCAAAATGCN	450
GGTGAAAGGN GAAACAAACA TANCATACCT ATGTTTCACG TTTCTATCNG	500
GCTCCNCGAA ACTAATTTTT TGGTGCCNCC NGATTATACC ACTTCCCATT	550
GATATCTGGT CNGCTGGCTG TGTCTTAANC AAAACTTCCT TTTGGGCCCN	600
CCTTTGTTTC CCTGGAAAAA AATGCCATNG AACCAACCTGT TAAAAATCNT	650
TCCNGGTTCN GGGAACACC NCNCCNTCA AAAAATCCCC NTNTTGAATC	700

CCCANTNTA CCAAATTCCC GGTTCCNCC GAAAAAANCC CNCCCTTGG 750  
 NNNAAGGTTT TCC 763

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 772
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Auxin-related gene

## (xi) SEQUENCE DESCRIPTION: SEQ ID:11:

GGTGAAACTT TACTTTGCA ATACACCGTC TAACAATGGC TGCAGCTCCA 050  
 AGTGAGTCCA TACCCTCTGT AAATAAGGCC TGGGTCTATT CAGAGTATGG 100  
 AAAAACTTCT GATGTTCTCA AGTTTGATCC AAGTGTGGCT GTTCCTGAAA 150  
 TTAAAGAGGA TCAGGTGCTG ATCAAGGTTG TTGCTGCTTC TCTTAACCCA 200  
 GTTGATTTTA AGAGGGCTCT TGGTTACTTC AAGGACACTG ACTCTCCCCT 250  
 ACCTACAATT CCAGGGTATG ATGTANCTGG TGTGGTGGTA AAGGTAGGAA 300  
 GCCAAGTAAC CAAGTTAACG GTGGGGGATG AAGTGTATGG GGATCTCAAT 350  
 GAAGACAGCA TTGGTGAACC CAACAAGGTT TGGGTCTTGT GCANANTACA 400  
 CTGCTGCAGA TGAAAGANTA TTGGCTCACA AACCCAAAAA CCTGAGCTTT 450  
 ATTGAAGCTG CTANCCTTCC CTTGGCTATT GAAACTGCC NTGAANGGCT 500  
 TGAAAGAACT GAACTTTCTG CTGGTAAATC CGTCCTTGTGTT TTGGGAAGCG 550  
 CTGGGGGTGT TGGAACACAN ATTATTCAGC TGCAAAGCAT GTTTTGGTG 600  
 TTCCAAAGTA GCAGCTACTG CAAGCANTAA GAAACTGGAT TTGTTGAGAA 650  
 CNTTGGGNNGC TGATTTGGCT ATCGATTACA CCAAGGAGAA NTTNGAGGAC 700

CTGCCAGAGA AATTGTGATGT AGTGTATGAT GCAGTTGGGG AGACAGATAA	750
GGCTGTGAAG GCGGTGAAAG AAGGCGGGAA GGTTGTAACA ATAGTAGGTC	800
CAGCAACGCC ACCGGCTATC CTTTTGTGC TTACCTCTAA AGGGTCTGTG	850
TTGGAGAAC TGAAGCCTTA CTTGGAGAGT GGGAAAGGTGA AGCCAGTTCT	900
TGATCCCACA AGTCCATATC CCTTTACTAA AGTTGTTGAA GCATTTGGTT	950
ACCTTGAGAG TTCCAGAGCT ACCGGAAAGG TGTTGTGTA TCCCATCCCA	1000
TGAGGTTGAG AGTGTATGTG TGAATGATCT ATGAGACTAT GATTGTGTAG	1050
AGTCCATTTC CTTCCTCTTG TATGTGTGTA GCAGTATATT TTAATCTTGA	1100
AGCCTTGTAA TAATGAATAA GATTGAGTCC TTAATAAATT GTCATTACAT	1150
G	1151

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1167
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Sucrose transporter

## (xi) SEQUENCE DESCRIPTION: SEQ ID:12:

CCATTGGCGA TCACGTACAG TGTTCCATAT GCCTTGATTT CTTCTCGTAT	050
CGAGTCTTTG GGACTTGGCC AAGGCTTATC AATGGGTGTA CTGAATCTGG	100
CAATCGTAGT ACCACAGGTG CTGGTATCCC TGGGAAGTGG ACCATGGGAT	150
CAGCTATTGG TGGTGGAAAC TCTCCAGGGT TTGCGGTTGC AGGAGTTGGA	200
GCCTTAGCAA GTGGGCTGGT GGCCAATCTT GGCTATTCCA CGTTCTATTTC	250
CACAGAAGCC TANATCTTTC ACATGAGGTA TTTTGTGTA TCTACTTTTT	300

ACCCAACCTT	GTCACAGAAA	TACAAAACCT	CCATAGATAG	TGAGAATTG	350
TAAATATCTT	TTGTTACGTG	TTAGCTATTT	CTCAATACAC	TCATTACCA	400
GAGGTTCTT	TAGTTCTGGA	AATTCTCTC	TTTCCCTTTT	TGTCGTTTTA	450
GATGCTTAA	TAAAGAAAGG	CCTGGCAGCG	ATTATATCAA	AGTTGANCTG	500
AATATCTGTG	TTGAAGTGCT	TCCGTTAAC	AATTATAGT	TCTCAATTTC	550
TACAATATT	TAAATCAGAA	CTGTCACCTG	GTGGACTCTT	ATGGAATCCA	600
TATGTTGGAA	CCATAATCTC	AATTAGGCAT	CGTGCCTCAA	TTCCACAAATG	650
GTGTTTCAG	AAGTGTGATG	AAACAAGTTA	GTCAAGAAAG	TGATGGTGT	700
TTCACAAATG	CTGGCTACGC	AACGATATTG	ATGTGGGTAC	GCAAATTGAT	750
TGATGTAGTA	GCCATCACTA	AGTTCTGGT	TAGACAAGTT	ATCTACAATT	800
AGTGGANAAT	TTCTTGAATG	AAAATCAGTC	CCATCTGGT	GATTGTGGCA	850
AATTGCTACG	GAAAAGTAGG	TGAAGCCTCA	GCTGTAGGAT	TTGGAAATT	900
CTTGAAGAGT	AGTCCCTAC	CAACCAGGAT	ATGTTCTGC	TTTCGAGAA	950
TTTGTCTCC	TGAAAATATC	GTTCCTCTT	TTGGCAAAGT	TGATTTGAC	1000
TTAGTGGTTT	AATCATGAGG	TATTGGAATC	TCATGCGTTT	TGTGCATGTA	1050
TTTGTANTAT	GAATGTGGTG	AAATGTGCTT	GGTGGCCAAC	AGTGAATATA	1100
TGAAATGTAC	TGATTGAAAC	CTTGATGGAN	ACATCCCTTT	TAATTGCTGT	1150
TTTGGAAAGCT	TGGGTCC				1167

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 476
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: meristem pattern gene

## (xi) SEQUENCE DESCRIPTION: SEQ ID:13:

CCTCANNAAAT	CTCTATATT	TTTGGGGGCG	TGGGTGGTCT	AANAATATGT	050
TCTTGGCTTC	AAAACCCTCA	TCAGATGGAG	AGCACCGACT	CGCTTCCGG	100
CTCGCAGGCG	CCGCCGCAGC	CAAACCTACC	TCCGGGATTC	CGCTTCCACC	150
CCACCGATGA	GGAGCTAGTC	GTTCATTAACC	TCAAGAAAAA	GGCCTCCTCG	200
GCTCCCCTCC	CCATTGTCAT	CATCGNCGAA	GTCGACCTCT	ACAAATTGAA	250
TCCATGGNAG	CTCCCAGAAA	AGGCGACGTT	CGGAGAGCAA	GAGTGGTACT	300
TTTCAGTCC	TAGAGACCGG	AAAGTACCCN	AACGGAGCAC	GGNCTAATAG	350
AGNAGGGACT	TCAGGNTTTT	GGTAGGGGAA	CCGTANTGAA	AAGCCCTTTT	400
GGGTTGNACT	ATTANGAGGN	NGGGGGNTCT	CCCAAANTTG	NGGTNAAAAN	450
GNANTTNNTT	NTTNANGGG	ACNNCC			476

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 497
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: transcribed sequence, T45086

## (xi) SEQUENCE DESCRIPTION: SEQ ID:14:

TNAATTAANG	GCAGCCNATT	CGGTGAATT	CCTTCATTG	ATCCTGCAA	050
CATGCCTTAT	GGNAACGCTT	GAAGTCCTTC	TGGTTGGGN	CAAAGACCTT	100
GNAGACCATG	ATTTTTCGG	TAAAATGGAT	CCCTATGTCC	TTTTATCATT	150
AAGGACCCAA	GAGAAGAAGA	GCACTGTGGC	ATCAGGACAA	GGATCTGCAC	200

CAGNANTGGN AATGAAACTT TTCAATTACAC AGTCTCATCA GATGATGTTA	250
CCGAACTCAG CTTAAAATC TATGACAAAG ATACCTTCAC CCCAGATGAA	300
TTTCTTGGAG GAAGCAACCA TTCCTTAGN AAACAGTGTT CATGGGAAGG	350
AAGCACTGAA CCGACTAAAT ACAATGTCGT CAATGAGAAT AATGAATATC	400
ATGGAGGATA TTACAGTTGG ACTCACTTTC ACCCGTGAAG CGAACCGGCT	450
CTCGTGCAGGG NGGNNTNTGAT GAAGAAAGAA CAA	483

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 488  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: transcribed sequence L36159

## (xi) SEQUENCE DESCRIPTION: SEQ ID:15:

AGGATATGTT GATTAGAACT CATGTAACCT CATATTACAC ATCTTAATAT	050
CTCCAATTAC ATGAACGTAA AATAAAACCC CTAACTTCCA CAAAGCATCA	100
ATCANACACG GGGNACGTCC GCGAATGCTA AGCAAATTGA CATCATCGAT	150
CACCGGACCA CACAGAGAGC CGGAGTGATC GCTCGTCATG GTGTACATTG	200
TGCTCAGAAA CATGACACGC GTGCGCGGCG NACACGGNGG TGNAAGAAGA	250
GCCTGGCCTT CTTGNAACCC TCCTTTGCCT TTGGACTCAT AAGGAACCTT	300
CACAGTCTCC TTGCCGGCAA ATGCCCTCGAT AAAGAGGGAG CCTTCGCAGT	350
CGTTGGTTCC CGTCGNCGAC AGAGAATNTN AGGGCGTAGC GCCTNNCGGG	400
NTTGGTGAAG ACCACTTGAG CCAATGNGCT CTCTTTCCC GGCAACGAGC	450
TCGNTNGGTN TTAGGCCTCC NGGANGGAA GTGTGGNG	488

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 460  
(B) TYPE: cDNA  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: transcribed sequence, T45902

## (xi) SEQUENCE DESCRIPTION: SEQ ID:16:

GTTTGTCTTC GGTTCTAAA GAGAGAGACA CCCAGAATTG GNTTCAGAAA 050  
TCGGAGATTA AGTTCTGAA CCAAGTTCAA GCCCTGAGA GCGTCGCCTT 100  
TGATCCACAA GGACGTGGAC CATATNCTGG TGTTGCGGAT GGGAGGAGTC 150  
TTGTTCTGGA ATGGGCAGGC CTGGACTGAT TTTGGCTATN CATGCCNCA 200  
CAGGTCAAGA TATATGTGNA TCCCANAACC ATCAGCTATG ANTTACTTGG 250  
CAAATGAGCA CATCTNTNGN AGGGGCCNTG GGGCTCCCCC TTTTGGNAAA 300  
GAAAACAGGA GATTGGNGC AATTGGGGG TTGAATACTT TTGGGCTTTN 350  
TTNGAAAATG GGGCAAANGN TNNGGTTTNG GGAAATTCC ACTTCNAACT 400  
TAGGANAANG GGGNGCCATG NGGGTTTCTT ACCCTCTTGG NNTGGTGAGG 450  
ANGGANAATT 460

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 480

(B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:17:

NTGGGTTCCA	TGACACTTCC	TAAAGAGCTT	CCCACCATCA	ATTCTCCCT	050
CCAAGACTTG	AAGCCTGGCT	CAAGCTCCTG	GACTTCCACC	TGCAAACAAG	100
TCCGCAATGC	ACTCGAAGAA	TATGGTTGCT	TTGTGGCATT	GTNCCCACAA	150
GTCTCCCAAG	AGCTCATGGA	CAGTATCTTC	GGNCAATCCA	GGGATCTGTT	200
CGAGGTTCCC	CTCGAGAACAA	AGGTCAAGAA	CACCAGCGAG	GAGCCTTACCC	250
GTGGNTATAT	CGGACCAAAC	CCCCTCTTGC	CACTCTATGA	AGGCATTGGC	300
ATTGACAACG	TCACATCCCA	ACAAGAAACT	CAGAAAGTTC	AGGGACCTCA	350
TGTGGGCTAA	TNGAAAGACC	CAATTCTGTG	AAAATCACAG	ATCTTGTGTTNG	400
GCANGTNGCT	CGGGGAGTTN	GGAAAACACT	GTGGAAANGA	TGNTNTTNCG	450
NAAGTTACGG	GNTACCTCTT	GGGGANNTNA			480

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 673  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:18:

GATTGGGTA	CANTTACAGT	ACCAGATATC	AATATCAATA	CTAGATAACA	050
GTATATNGCA	CGTCCTCTTC	TTCTTCTTCT	TCTTCTTCTT	CTTTTTGGT	100
GGAAGCTCGT	CTTCTTCTTC	TTCTAGCTAG	CTTCTTCAG	CTTTTTTAT	150
TTGTTATTCT	TCATCTCTA	CCCTAATATA	CTCTTGTATA	CATAAAAGTC	200

CAGCACTTT	CAAACAATAG	CAACTCAGTA	GTCTTACCC	TCAGTAGTGA	250
TTAAAAACTA	CTGCGTCGTC	ACTCCACAAG	AGCTTGTATT	ACCACNTAGA	300
TGGCCTCATT	GCGCTCTCTC	GCATTCCAGG	TGAATCACTT	CGAGCTGCAA	350
CTTATAACGC	CGGCAAAGNC	AACACCGCTC	GAAATGAAGC	TGTTGGTCGA	400
ATATCGACGG	ACCAGCAATG	CCTCAGGTCT	CATGTTCCCC	ATTCATCATG	450
TCTTACAAGA	ACAATCAATC	AATACTGTCT	GAAACCAAAC	GACCCGNNGG	500
AGGTGGATTA	GGGGATGCGC	TGAGCAAGGG	ACTGCAGTTT	TACTACCCCT	550
TGGGTGGTNG	GTTCANGNG	GGGCCTAACCA	AAAGGNTATG	GNNGACTGAA	600
CCGNGAAGGA	ACTTGGTCGN	TGGGGGAACG	CCGAGGCAAA	NCGAGGACTC	650
GGGNTGAACC	CANCGCCNNGG	CCA			673

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 749  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:19:

AAATTGAGGT	CAGTATAAAAT	TCCAAACACA	CCATCAAACC	ATCAACTTCC	050
TCTACACCAC	TTCAGCCTTA	CAAGCTTACC	CTCCTGGACC	AGCTCACTCC	100
TCCGGCGTAT	GTCCCCATCG	TATTCTTCTA	CCCCATTACT	GACCATGTCT	150
TCAATCTTCC	TCAAACCCCTA	GCTGACTTAA	GACAAGCCCT	TTCGGAGACT	200
CTCACTTGT	ACTATCCACT	CTCTGGAAGG	GTCAAAAACA	ACCTATACAT	250
CGATGATTIT	GAAGAAGGTG	TCCCATAACCT	TGAGGCTCGA	GTGAATTGTG	300
ACATGACTGA	TTTCTAAGG	CTTCGGAAAA	TCGAGTGCCT	TAATGAGTTT	350
GTTCCAATAA	AACCATTAG	TATGGAAGCA	ATATCTGATG	AGCGTTACCC	400
CTTGCTTGGG	GTTCAAGTCA	ACGTTTCGA	TTCTGGAATA	GCAATCGGTG	450

TCTCCCGTCT	CTCACAAAGCT	CCATCGATGG	AGGAACGGCA	GAATGTTTTC	500
TCAAGTCCTG	GGGTGCTGTT	TTTCCGAAGG	TTGTCCGTGA	AAATATCATA	550
CATCCCTAAT	CTCTCTTGAA	AGCCAGCATT	GCTTTTCCCC	ACCGAAAANA	600
TGACTTGCCT	GAAAAGTTAT	GCCGATCAGA	TGGAAGGGTT	ATGGTTTGCC	650
CGGAAAAAAA	TTGCTACAAG	GAAATTGTGA	TTTGGTGTNA	AAACCATATC	700
TCCATTCCAG	AAGAAACGAA	AACGANTCCG	TGCCCAAGCC	ATCACAATT	749

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 218  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:20:

TCGGGCAGAG	AAATCTTTGA	GATTGGCAGA	CTCGAGAGCA	TCCAGACTTC	050
GAGAAAGAGT	AGAGGGAGCTT	ACCTGTCAAC	TGGAAGAATT	TGAAAATCGG	100
GAGGACTTAA	GGAGAGGCCT	GGGTGGACCT	AGATATGTAT	GTTGGCCCTG	150
GCAGTGGCTT	GGGCTGGACT	TTGTAGGGTT	CAGTCGCTCT	GATACAGAAC	200
AACAGAATAG	TTCAAACG				218

## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 437  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID:21:

CGCTGCTTGT	CTCGCTGCCT	ACTATCACTA	CTACCATGGG	TTGGTCCCCT	050
TTCCCTTCAGA	ATCGGACATG	TTTTGGGACG	TTCAGATTCC	ATCTATGCCG	100
CTGTTGAAGT	ACGATGAGGT	ACCCAGCTTC	TTGTACCCCTA	CTAGTCCTTA	150
CCCGTTTTTG	AGGAGGGCCA	TTTTGGGACA	ATACGGGAAC	TTGGAGAAGC	200
CCTTCTGTAT	ATTGATGGAC	ACTTTCCAAG	AACTCGAGAG	CGAGATCATC	250
GAGTACATGG	TTCGTTGGT	GCCCCATCAA	NGTTGTTGGT	TCCCCCTTCT	300
TTCAAAGAAC	CCCAAAAGCC	CAAAANCCT	NTTCCCCCGG	GGGATTTCCA	350
TNAGGGCCGA	CGNANTTCAN	CCANCCGGTT	NGTTTCGGAA	ACNAAAACNN	400
AACANNTTTC	GNGGNTTTTT	NACACCCANG	NTNNCGG		437

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	232
(B) TYPE:	cDNA
(C) STRANDEDNESS:	Single
(D) TOPOLOGY:	Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID:22:

AAGAAAGGAG	TCTCGTCAAT	AAAGGATTG	TGAGAATCAA	ATAACGTTCT	050
CTGTTTATTA	ATTTGTAACA	GTAGTTGAT	CGAGTCTGTG	AGTAAGTGAT	100
CGAGTAAGAG	ATGTACTCTA	CTGTGTGTG	GTCAATCATG	TTCGTGTCT	150
TTGGTAGCCA	TGTAATGTTC	TCCATCTGGT	CATTATCTGT	GGCCTTGTGA	200
TCATGTTTAA	TCAATGAAAC	TACTATTAGT	AT		232

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 469  
(B) TYPE: cDNA  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:23:

GATCGGTCCG ATGACCGGAA AAGTCATGAT TTTGAGGTGA GGAGCGANTT	050
GGGTTTCGCC NGAAATGTNC AAAGCCCTGT GCCTTCGGAG CATGTGGTTG	100
AGAATTGGGN GAAAGGCAAA GTGGGTGTCC AAGAAATTGG NGAANTTGGN	150
AGCTTTGATA AGGATTGGG ATAANTTCTN GTTTGATTCC CGCCNGAGAA	200
AGCTCGNTCT TCTTTGAAA TTTGACAANG AGGAGGGGTT CANCNCNAGT	250
CCAACAANNG AATCAAGGGA GGANANACTC ANCTTNAGAC TCANCCTTCG	300
CNCAGANGNA GNAANNTAAA AACTGNGGCG AAAACCGNCT NNCGAGGTGA	350
TAATTAANNT CCACCTTCTT TNTTNCACGG TCCCCCGCT TNTTTTTNNNA	400
GCTTTTTCTC CNTCAANGCN AATTCCCGTT NGNTNTTCTT NTTNTGCCNA	450
NNCTAATNCN CTTNATTCC	469

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 178  
(B) TYPE: cDNA  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:24:

AACCAGATAT	NAAGCGATT	TCGATATTCA	ATAACATTCT	TCTTTAACTG	050
TTCAGGTGCG	TCAGGAGCCC	AACGCTCAGG	GTAATCGGCG	AAAGTGAATN	100
TTGGNTNGAC	ATTAGNAACC	AGCCAGACCA	ATAGCCGTTG	GAACAGCTGA	150
CGTTCGGCCGC	GCCCAACCAGG	TGGNGCAA			178

## (2) INFORMATION FOR SEQ ID NO:25:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 244
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:25:

TTCAAGTTAA	CCTCTCAAAC	CCGACACAGA	GAGCATAAAAT	GGGTTCCGAA	050
TCATTGGTTC	ATGTTTTCTT	GGTTTCCTTC	ATCGGCCAAG	GCCATGTGAA	100
CCCACTCCTC	CGNCTCGGNA	AGCGCCTCGC	TGNCAAAGGT	CTCCTCGTCA	150
CCTTCTGCAC	CGTCGAATGC	GTGGTAAGG	AAATGCGNAA	GTCCAACGGC	200
ATCACCGACG	AGCCAAACC	AGTTGGAGAT	GGATTCAATCC	GCTT	244

## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 685
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:26:

CCAATTGGT	CGCCGTAAAA	CATGGTTAAT	CAAACGGTGA	ACGGAAGCCA	050
ATCAAGTAGC	GGAACCCAAA	AGCTCAATGC	TTCAAGCAAC	ACCAAGAGGG	100
ATTTGAGGC	TGTGAGTGAG	TCCATGCACT	CTGCAATTTC	AATGAGTAAA	150
ACAGAAGTCT	TGGATTCTGT	GCTGAGTGAT	TTCTCTGAGG	GATATTAG	200
CCTTGCTAT	GAGAACGTC	GAAAATTGCT	TGTGCAACTT	GCCAAAGAGT	250
ATGATCTTAA	CAGGACNCAG	GTTCGCGATT	TGATAAAGCA	GTATTGGGA	300
CTTGAGCTTC	CTGGAACTGG	AAGTGACAAT	GCTGACTCAG	AAAGAGGAGG	350
CATCTCTTTC	TGCTTTCTAC	CGCATTGANA	GGAACTTGAA	GACNTGCTCT	400
CNAGCCCAGT	TATGAANTGC	TATTTGAGCG	GCTTAATAACG	CNTCCGGAG	450
GGTTGAAGTT	CTTGTCTATT	CTTTCGAGCT	GATATCTTTA	TCCATTCTCG	500
CANAAAAATA	ATCTGGCGTC	TTTGCNAACA	TTGGATTCCC	CATTCAAAGG	550
AGAAACTTAN	TNCGTTGGTT	AATCCCCCTG	CCTTANNAGC	TCCNCCCCA	600
TCNCTCGGAT	GATTCTTCCT	CCCTTTGCTG	GGAAAAAATT	GTNGCTTACT	650
AAGGCCGTGC	TTCCCATCCA	NCTATTCTTC	TNGAT		685

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 668  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID:27:

AAGTTCACCC	AAGTATAAAG	TCTATCTTAT	TATATATAACG	TTTTCTACAA	050
TGTTTGACTA	TTACTGATAT	TANAANATCA	GCTTAAGGAG	CAACAAACAT	100
ATTATTACAT	TATAATGACA	ACAGTACATT	GATAATCACT	TTCCACTATA	150

GAAAACAACA	AAATTAAAAG	TGTGGACACA	TCCGTTATTA	CATTGCTACC	200
CGGCTATTCT	GTTGTATTCTT	GAGGTTCCCTT	CAGTGGCTCA	ACGTAACGGG	250
AAAGTACATT	AAAANTATGG	ATATGCCCTG	TNCTGAAATA	TGACTGAAAA	300
TAATCTTCAA	TGTTGCCCAA	TCTGTAAACA	TAGTTCACCA	TGATACCTCC	350
ACTTTGATNA	AGGCCTTTAT	CTGATCGATC	AGCCATCCNA	TTAATTCTCT	400
CAACCATTGC	TCCATTCTGT	NAGTTGAAAA	TTTGCACACAG	AATCCANAAC	450
TTTGCCTCTC	TTTTTCTCTT	GCAAAAANGT	ANCTGGCACA	CAATCCCATT	500
AAAAAGGGGT	TTTTAGAACT	GAAAACCAAT	TTATCANAAC	TTTGTTCCTT	550
CCCGGGTTTG	CTGAANTTCC	GTAAATTGAN	CATCCCTCCA	TGCCGTTTTT	600
TCCCCNTGGG	TGAATTCAAA	AAACCTNCTC	NAAAANTNTT	TCTAAAACNG	650
GCGCGGGGCC	ATNCATTIT				668

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 522  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: O-methyl transferase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:28:

NTNNNGGGGT	TGGGGNTCTN	GAAGGCAAAA	GATTCGGTCA	GGACAAGGTC	50
CTCGTCGAGA	GCTGGTATCA	TTTGANGGAT	GCAGTTCTTG	ATGGTGGGAT	100
TCCATTTAAC	AAGGNCTATG	GCATGACTGC	ATTTGATTAC	CATGGNAACT	150
GACCCTAGCA	TTCAACAAAGG	TCTTCAACAA	GGGAATGGCT	GACCACTCCA	200
CCATTACCAT	GCANGTAAAA	TCCTTGTAGT	ACTTACAAAG	GCTTCGAGGG	250

50

CCTCAAATCC ATCGTTGTAT GTCGGTGGCG GNACCGAGC TGTGGNGGAA	300
CATNATCGCT TCCCNAAGTN CCCTTCGCAT CAAGGGTCAT CANCCTTCG	350
ACTTGCCCTC AATCTTANTC GAANGCATTG CTCCNTCAAT TATCCTNNNT	400
GTTTCCANCC ANGTTGGGAT GNGGGGANAA TCTTCTGGCN ANNTCTTACC	450
CAATTNNNGGN ANNCTTCCAT TCTTCCCCT TTNAGTTCTNT NTTTINCTCA	500
ACCTAACTTG NCGNTCCNTC GN	522

(2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 445  
(B) TYPE: cDNA  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Acyl carrier protein

(xi) SEQUENCE DESCRIPTION: SEQ ID:29:

ATGGCCACCA	CCACAGGGAGC	TGCTTCTTCG	ATCTCACTCC	GCTCTEGCCT	50
TCACCAGAAT	CTTGCATTGT	CCAGGGTCAA	TGGTCTTAAG	CCAGTTTCAC	100
TGTCTGGTAA	TGGAAGAAGT	TCTCTTTCTT	TCGGGTTACA	GCAGCGTTCA	150
GTACGGCTTC	AGATTTGCTG	CGCGGCCAAA	CCAGAGACAG	TGGACAAGGT	200
GTGCCAGATA	GTTAGAAAGC	AACTTGCTT	ACCATGAC	TCAGCAGTTT	250
CTGGAGAGTC	AAAATTTCT	GCACCTGGAG	CTGATTCTCT	TGATACGGNN	300
GGAGATTGTG	ATGGGACTTG	AGGAGGAATT	GGGTATTAGT	GTGGNNNGAGG	350
AGAGTGCTCA	GAGCATTGAA	CTTNTNCAAG	NTGCTGGGT	CTTTTCNANA	400
AGNNNCATNG	NAAGACCAGG	NTTIGGAGGA	GGANTNANA	ACAAG	445

## (2) INFORMATION FOR SEQ ID NO:30:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	562
(B) TYPE:	cDNA
(C) STRANDEDNESS:	Linear
(D) TOPOLOGY:	Single

## (ix) FEATURES

(D) OTHER INFORMATION:	Elongation factor 2
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## (xi) SEQUENCE DESCRIPTION: SEQ ID:30:

GGATCATCCC TTGGNCCAAT ACGACCATCA TCAATGGNCT CAGGAAGACC	50
TTCCTCCAAC GGGNGTGCTT CCATGTACAG ACGGTTGTGC TTGTTGGGAG	100
ACTTGCTCAT CACAGTACGG NAGGNCTTCT CAAGGACTGT CTCACGGNAG	150
GACACAACAG GATCAGATT TACAATTCC GCTCCACCCA TAAAATCATC	200
TTGNAGATCC NTCANGNAGA TCTCAAGGTG AAGTTCACCA GCTCCAGCAA	250
TGATGTGCTC TCCAGACTCC TCAATGGTAC AGACACCCAT AGGGATCGGG	300
TCTTAGCCAG ACGTTTCAGC CCTTCAACAA GCTTGGGGAA GGATCAAGAA	350
GCANCCTTAC GNTTGAACAG CAACACGCAC AACAGGGTTG AGACAGGAGA	400
ACTTCATTGC ACGAATGGGG GGAGCATCTT GTTNCCTCT CATTGGTCA	450
AGGTAGCATT CTTGGGTGGA TTGAACITNT TCCCAGACCA ACCAAGGGNA	500
CAAGTTTTTA CCACAGGGGA ACATCCTCAA CAGTTTCNTT GTTTCTTTTC	550
CCCATCCAGG TT	562

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	490
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(B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

(ix) FEATURES

(D) OTHER INFORMATION: Auxin-induced mRNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID:31:

ATCGACTGCA TTAAGTTGCT AGAAGTGGAG CTTGGTGACA AGCCTTTCTT	50
TGGCGGTGAG ACCCTCGGAT TTGTGGACGT GACGCTCGNT CCTTTCTATT	100
CCTGGTTCTC TGTGTATGAG AAATACGGCA ACTTCAGCAT TGCGCCAGAG	150
TGCCCAAAGT NCATGGCTTG GGTTAACAGGG TGTATGGAGA AGGAGAGTGT	200
GTCAAAGTCT CTTCCCTGACC AGGACAAGGT CTGTGGCTTN GTTGCCGAGA	250
TGANGAAGAA GCTTGGAGTT GAGTAGATGT GATCAATGTC ATNTTGATCA	300
TGTCCTTGTT TTAGCCCCAA GATTCANCCT CGTTTTGGGT TGCTTGTTATT	350
TTTCAATAAA ATTGGGGGAC TTGGACCAAG CCCTCCAATA GTAGGAAGCA	400
CTCTTTCNGT GCCTCTTGGT CCNGT1TTTC TTCNGNTAAN CCTNTNTGCA	450
GCTAAAATTC ACCGNATTNC TGNTTTCCCT NTATNGCCAA	490

## (2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 483  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

(ix) FEATURES

(D) OTHER INFORMATION: Cysteine (thiol) proteinase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:32:

GGATCTCCTC	CTCCTCTCTC	TCCTTCTCCT	CCTCTCCTCC	GCGTCGCCT	50
CCACCGTAAC	CGACGCCGGC	GATCCTCTCA	TACGACAAGT	CGTACCGGGC	100
GCGGCCGAGG	ATGACGAGCT	CCTCCACGCG	GAGCGTCACT	TCTCGAACTT	150
CAAAGCCACG	TTCGGAAAGA	GCTACGCGAG	CCAGGAGGAG	CACGACTACA	200
GGTTCCGGCG	TATTCAAGGN	CAACTCCGCC	GGGCGAAGAG	GCACCAGGGG	250
CTTGGACCCC	ACCGCCGTGC	ACGGTGTCAA	CGAAATCTCC	GATCTCACTC	300
CCAAGGAGTT	TCGNCGGGAA	TTTCCTCGGG	CTTAAGAAGG	GGTCGGANTT	350
CGGGTTACCG	GCCGACGGTT	AAAAAAAGGGG	CCNGATNCCT	NCCGGANGAA	400
TTANCTTCCC	CACCCANTTT	TGGNNNTTGGG	GNGAAAAAAG	GNGCCCGNCN	450
AAGNCGGNGG	AANGGNCAAG	GGGGAAATNG	GGT		483

## (2) INFORMATION FOR SEQ ID NO:33:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Cellulase (endo-(1,4)beta-n-glucanase)

## (xi) SEQUENCE DESCRIPTION: SEQ ID:33:

ACGGTGGGGG	GACAGACTAC	CTCCTGAAGG	CCACGGGGGT	TCCTGGCGTC	50
GTCTTCGTCC	AAGTCGGCGA	CCCATACTCC	GATCACAACT	GCTGGGAGGA	100
GGCCGGAAGT	ACATGGTACA	CACGCCGCAC	GGTGTACAAA	ATCGACCACA	150
ACAACCCGGG	ATCCGACGTG	GNAGGTGTAA	ACCGCAGTTC	GTGCTCGCCG	200

TCGCCTCTAT CGTTTCAGG TCACGTGACC CCGCTTACTC GNAGNACTGC	250
TTCTCAATCG GAGCCGTTAA GGTTT1CGAG TTCGCTGATA CCCACCGTGG	300
TGTGTTCAGA TCCAGCCTCA AAAACGCCGT TGTGCCCTT TTTTACTGTG	350
NAANGTCAA CGGNTTCCA GGGATNAATT TACTNTTNGG GGAGGNAGCG	400
TTTGTGNN ACAAAAGGTGG TCTATTNGC NGGAGTACAA GTAGTATTNT	450
CATTGTGNTN AATCGGANGN CTATTTGGG GGAGNTTTNA GGNTNCMT	500
TAANGAANTT TGNNTGGGCT	520

## (2) INFORMATION FOR SEQ ID NO:34:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 695
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Pyruvate decarboxylase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:34:

GGCATTTT CACTCGAAGT CTCAATTTT CATCACAAAC ATTCCCATT	50
GATCACAAA AAGTTTCAAC CTTTAAACCT CCATGGACAC CAAGATTGGC	100
TCCATCGACG TCTGAAAC CGAGAACAC GACGTCGGTT GTTTACCAAA	150
CAGCGCCACC TCCACCGTTC AAAACTCAGT CCCTTCGACC TCCCTCAGCT	200
CCGCCGACGC CACCCCTGGC CGCCACCTGG CACGCCGCCT CGTTCAAATC	250
GGCGTCACCG ACCTCTTCAC CGTCCCCGGC GACTTCAACT TGACCCCTCT	300
CGACCACCTC ATCGCCGAGC CCGGCCTCAC CAACATTGGC TGCTGCAACG	350
AGCTCAACGC CGGGTACGCC GCCGACGGCT ACGCGCGGTC GCGTGGCGTC	400
GGCGCCGTTG CGTGGTGACT TTCACTGTTG GTGGACTGAG TGTGCTGAAC	450

GCGATCGCCG GCGCGTTATA GTGAGAATTG GCCGGTGATT TGTATTGTTG	500
GTGGGCCCA ACTTCTAATG ATTATGGGAC TAACCGGATT CTTCACCATA	550
CTATTGGGTT GCCGGACTTC ANTTCAAGAA CTCCGGTGGT TTCAAGAACN	600
TGACTTGCTT TTCAGGCTGT GGGTGAATAA TTCTTGGAAAG AATGCACATG	650
AATTTGCTTG AATACNGCAA TTTTCAATNG CNTTNGAAAN AAAAC	695

## (2) INFORMATION FOR SEQ ID NO:35:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 695
- (B) TYPE: cDNA
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ix) FEATURES

- (D) OTHER INFORMATION: Chalcone reductase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:35:

GGCCCAAATC CCAGAAGTGG TTCTTGAATC CTCCAACGGC CGCAGAACCA	50
TGCCTGTGCT TGGATTGGC ACAGCATCCA ACAATTACA ACCGGAGGTT	100
TTGATAGAAG CTGTTCTGA GGCCATCAAG CTTGGTTACC GACACTTCGA	150
CACTGCTTCC ATTTACGGCT CCGAGCAGAC TCTAGGAGTA GCCATTGCC	200
AAGCGCTCAA ACTCGGCCTC GTGGCTTCTC GTGACGAGCT CTTCATCACT	250
TCCAAGCTTT GGCCTAATGA TGGTCACCCC AACCTGGTTA TTCCCTGCTCT	300
CAAGAAAATC GCTTCAGAAT CTTGAGTTGG AGTACCTTGA TTTGTATCTG	350
ATACACTGGC CCATCAGTGC CAAGCCTGGG AAAGTTGAGT CACGCACTAG	400
AGGGAGAAGG ACCAAATGCC GATGGACTTC AAGGGTGTGT GGGCAGACAT	450
GGAGGAAGCT CAGAGACTTG GCCTCACCAA ATCCATTGGG AATCAGCAAT	500
TTCTCTACCA AAAAGACTCA GAATTTGCTC TCCTTTGGCT ACTATTCCCTC	550

CGTCAGTCAA TCAANTTTAA NATGANTCCA TTTTGGCAAC AGAAGAACCT 600  
 CAAAAACTTC TGCAAGGCCA GTGGTATAAT TTGTGACTGG CTTCTCCCCA 650  
 TTGGGTGCCA TNNGAACCAN TTGGGGGCAC CAATCATGTT CTCNA 695

## (2) INFORMATION FOR SEQ ID NO:36:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 765  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Protein kinase

## (xi) SEQUENCE DESCRIPTION: SEQ ID:36:

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GGGCANANCG TGGTGTGGGA ACTGGGTCAT TTGGAATTGT ATTCCANGCG 50  
 AAATGCTTGG AAACTGGTGA GACTGTGGCC ATAAAGAAGG TTTTACAGGA 100  
 CAGAAGGTAT AAGAACAGGG AACTTCAATT GATGCGCGTA ATGGATCATC 150  
 CAAATGTGAT TTGTTTGAAG CATTGTTCT TCTCTACAAC AAGAAAAAT 200  
 GAGCTTTTC TCAATTGGT TATGGAATAT GTTCCGGAAA CTATGTATCG 250  
 GGTTATAAAG CATTACAGCA ATGCAAACCA GAAAATGCC CTTGTCTATG 300  
 TCAAACTTA CATGTNCCAC ATTTTCAGAG GGCTGGCTTA CATAACACACC 350  
 GTTCCTGGAG TTTGCCATAN ANATTGAAAN CCTCCAAATT TATTGGTTGA 400  
 TCCTCTTATT CACCANGTCA AGCTTTGTTG ATTTTGGAAG TGCCAAAATG 450  
 CNGGTGAAAG GNGAAACAAA CATANCATAC CTATGTTCA CGTTTCTATC 500  
 NGGCTCCNCG AAACTAATT TTTGGTGCCN CCNGATTATA CCACCTCCCA 550  
 TTGATATCTG GTCNGCTGGC TGTGTCTAA NCAAAACTTC CTTTTGGGCC 600  
 CCCCTTGTT TCCCTGGAAA AAAATGCCAT NGAACCACCT GTTAAAAATC 650

NTTCCNGGTT CNGGGGAACA CCNCNCNTT CAAAAAATCC CCNTTTGAA	700
TCCCCANTTN TACCAAATTTC CCGGTTTCCN CCGAAAAAAN CCCNCCTTT	750
GGNNNAAGGT TTTCC	765

## (2) INFORMATION FOR SEQ ID NO:37:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	772
(B) TYPE:	cDNA
(C) STRANDEDNESS:	Single
(D) TOPOLOGY:	Linear

## (ix) FEATURES

(D) OTHER INFORMATION:	Auxin-related gene
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## (xi) SEQUENCE DESCRIPTION: SEQ ID:37:

GGAGAAACCT CTGCCCTTA AACTTACTT TTGCAATACA CCGTCTAACAA	50
ATGGCTGCAG CTCCAAGTGA GTCCATACCC TCTGTAAATA AGGCCTGGGT	100
CTATTCAAGAG TATGGAAAAA CTGCTGATGT TCTCAAGTTT GATCCAAGTG	150
TGGCTGTTCC TGAAATTAAA GAGGATCAGG TGCTGATCAA GGTTGTTGCT	200
GCTTCTCTTA ACCCAGTTGA TTTAAGAGG GCTCTGGTT ACTTCAAGGA	250
CACTGACTCT CCCCTACCTA CAATTCCAGG GTATGATGTA GCTGGTGTGG	300
TGGTAAAGGT AGGAAGCCAA GTAACCAAGT TTAAGGTGGG GGATGAAGTG	350
TATGGGGATC TCAATGAAGA CAGCATTGGT GAAACCCAAC AAGGTTTGGG	400
TCTTTGGCAG AGTACACTGC TGCAGATGAA AGANTATTGG CTCACAAACC	450
CAAAAACCTG AGCTTITATTG AAGCTGCTAA CCTTCCCTTG GCTATTGAAA	500
CTGCCCATGA AGGGCTTGAA AGAACTGAAC TTTCTGCTGG TAAATCCGTC	550
CTTGTGTTGG GAAGCGCTGG GGGTNTTGGG ACACATATTA TCANCTTGCC	600
AAAGCATGTT TTTGGTGCTT CCCAANTAAC NNCTACTGCA ANCACAAAAA	650

AACCGGAATT	TGTTGAAAAA	CCTGGGTNCT	GATTTGGCTA	CCAATTACCC	700
CANGAAAACT	TCCAAGAACT	GCCCCAAAAAA	TTGAATTITN	TTTTTNANGC	750
CNTTNGGGAA	ANNAANAAGG	GT			772

## (2) INFORMATION FOR SEQ ID NO:38:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 773  
 (B) TYPE: cDNA  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (ix) FEATURES

(D) OTHER INFORMATION: Sucrose transporter

## (xi) SEQUENCE DESCRIPTION: SEQ ID:38:

CATTGGCGAT	CACGTACAGT	GTTCCATATG	CCTTGATTTTC	TTCTCGTATC	50
GAGTCITTGG	GACTTGGCCA	AGGCTTATCA	ATGGGTGTAC	TGAATCTGGC	100
AATCGTAGTA	CCACAGGTGC	TGGTATCCCT	GGGAAGTGGA	CCATGGGATC	150
AGCTATTGG	TGGTGGAAAC	TCTCCAGCCT	TTGCGGTTGC	AGCAGTTGCA	200
GCCTTAGCAA	GTGGGCTGGT	GGCCATCTTG	GCTATTCCAC	GTTCTATTCC	250
ACAGAAGCCT	ANATCTTCA	CATGAGGTAT	TTTGTGTAT	CTACTTTTTA	300
CCCAACTTTG	TCACAGAAAT	ACAAAACCTC	CATAGATAGT	GAGAATTGT	350
AAATATCTTT	TGTTACGTGT	TAGCTATTTC	TCAATACACT	CATTTACCAG	400
AGGTTTCTTT	AGTTCTGGAA	ATTTCTCTCT	TTCCCTTTTT	GTCGTTTTAG	450
ATGCTTTAAT	AAAGAAAGGC	CTGGCAGCGA	TTATATCAA	GTTGANCTGA	500
ATATCTGTGT	TGAAGTGCTT	CCGTTCAACA	ATTTATAGTT	CTCAATTCT	550
ACAATATTAA	AAATCAGAAC	TGTCCCTGG	TTGGACCCCTA	ATGGAATCCA	600
TATGTTGGAA	CCATAATCTC	AATTANGCAT	CCTGCCTCAA	TTCCNCAATG	650

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GTGTTTCAN AANTGTTGAN GAAACNANTT NNTCCAAAAA GTTGATGGTG 700  
TTTTTCCCAA ATGCCNGGCT ACNCCACCAA NNTTGANGTT NGGTACNCCA 750  
AATTGAATNA AGTTATTACC CAC 773

## CLAIMS

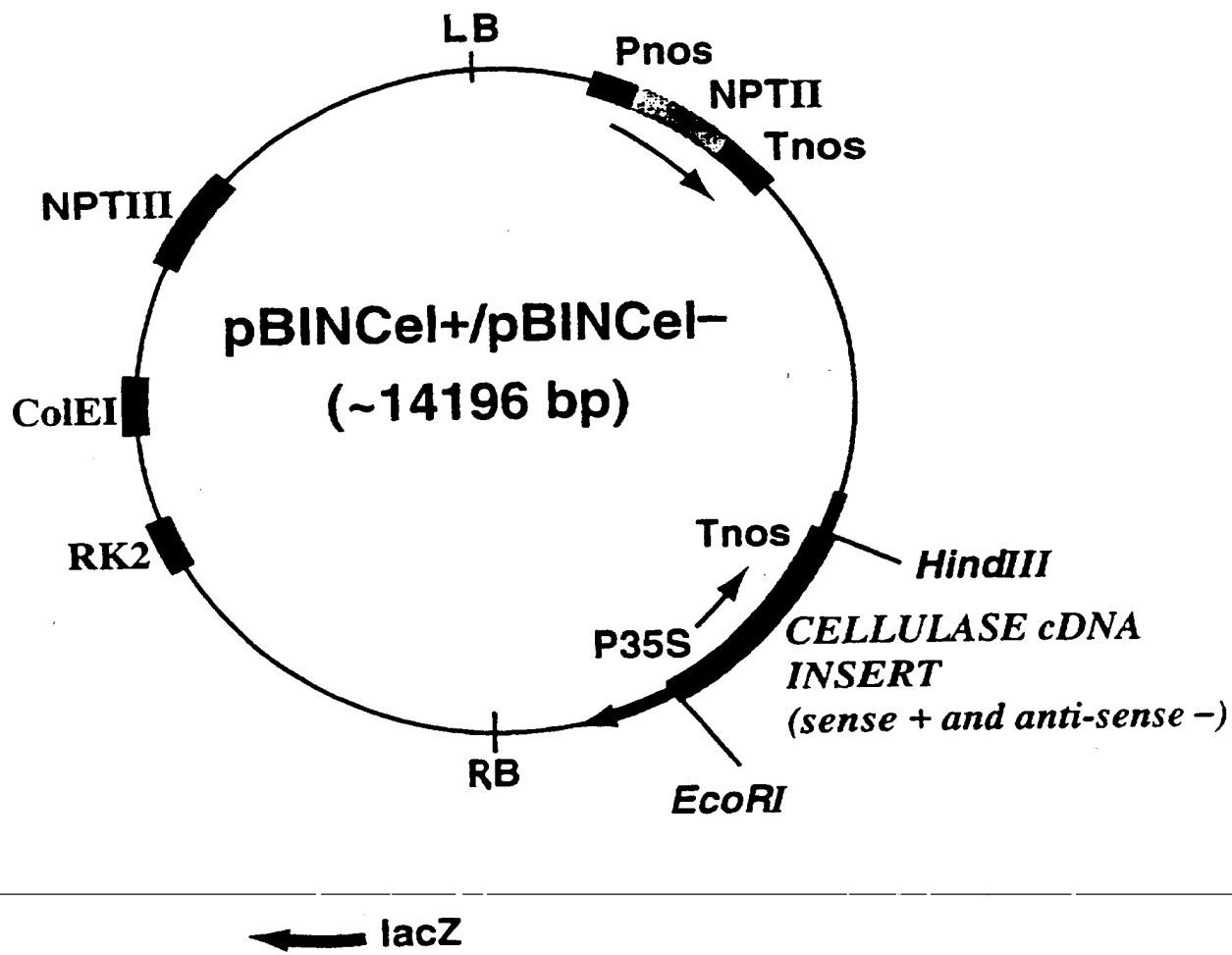
1. A vector for use in the genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region, or a fragment of at least 10 bases thereof, of a strawberry protein selected from O-methyl transferase, acyl carrier protein (ACP), elongation factor, auxin-induced gene, cysteine(thiol) proteinase, cellulase, starch phosphorylase, pyruvate decarboxylase, chalcone reductase, protein kinase, auxin-related gene, sucrose transporter, meristem pattern gene, or selected from a strawberry protein with homology to transcribed sequence, T45086, transcribed sequence accession number L36159 or transcribed sequence accession number T45902, or selected from a strawberry protein of unknown homology encoded by one of the StrawRipe sequences A to K.
2. A vector according to claim 1, wherein the regulation sequence comprises a sequence selected from SEQ ID NO:1 to SEQ ID NO:38, and fragments thereof with at least 10 bases.
3. A vector according to claim 1 or 2, wherein the regulation sequence is aligned for antisense expression.

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4. A vector according to claim 1 or 2, wherein the regulation sequence is aligned for sense expression.
5. A vector according to any preceding claim, wherein the regulation sequence fragment comprises at least 35 bases.
6. A method for genetic modification of a strawberry comprising inserting a vector as claimed in any preceding claim into the genome of a strawberry plant.

7. Propagation material for a strawberry plant which plant is progeny of a strawberry plant which has been modified by a method according to claim 6.
8. Strawberry fruit of a strawberry plant grown from propagating material according to claim 7.
9. Strawberry fruit according to claim 8, with regulated ripening in comparison with unmodified fruit.
10. A gene regulation sequence selected from SEQ ID NO:1 to SEQ ID NO:38, and fragments thereof with at least 10 bases.

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## **INTERNATIONAL SEARCH REPORT**

Int'l Serial Application No.

PCT/GB 97/00178

A. CLASSIFICATION OF SUBJECT MATTER					100, 200, 300, 400, 500, 600, 700, 800, 900
IPC 6	C12N15/11	C12N15/82	C12N15/52	C12N15/54	C12N15/55
	C12N15/56	C12N15/57	C12N15/63	C12N9/10	C12N9/14
	C07K14/415		A01H5/00		
According to International Patent Classification (IPC) or to both national classification and IPC					
B. FIELDS SEARCHED					
Minimum documentation searched (classification system followed by classification symbols)					
IPC 6 C12N					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
Y	PLANTA, no. 194, June 1994, BERLIN, pages 62-68, XP000197143 MANNING K.: "Changes in gene expression during strawberry fruit ripening and their regulation by auxin" cited in the application see the whole document --- PLANT MOLECULAR BIOLOGY, vol. 6, no. 27, 1995, DORDRECHT NL, pages 1097-1108, XP000670213 WILKINSON J.Q. ET AL.: "Identification of mRNAs with enhanced expression in ripening strawberry fruit using polymerase chain reaction differential display" see the whole document --- -/-/				1-10
Y					1
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.			<input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents :			*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search			Date of mailing of the international search report		
16 April 1997			24.06.1997		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016			Authorized officer  Panzica, G		

## INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00178

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 10622 A (ZENECA LTD.; GB) 20 April 1995 see the whole document ----	2-10
A	WO 92 12249 A (MONSANTO CO.; US) 23 July 1992 ----	
A	WO 91 16440 A (IMPERIAL CHEMICAL INDUSTRIES PLC; GB) 31 October 1991 ----	
A	HORTICULTURAL REVIEWS, vol. 17, 1995, NEW YORK US, pages 267-297, XP000197328 PERKINS-VEAZIE P.: "Growth and ripening of strawberry fruit" -----	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 97/00178

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: 1-10 because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
Claims 1-10 of invention 1 have been searched keeping Seq. Id. No. 1 and 28 as subject matter, since the concept defined as "O-methyl-transferase" is vague and too broad.
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

27 inventions \* see continuation-sheets PCT/ISA/210 \*

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 (partially)

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 97/ 00178

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry O-methyl-transferase and its use.

2. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry acyl-carrier protein (ACP) and its use.

3. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry elongation factor and its use.

4. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry auxin-induced gene and its use.

5. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry cysteine(thiol) proteinase and its use.

6. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry cellulase and its use.

7. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry starch phosphorylase and its use.

8. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry pyruvate decarboxylase and its use.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 97/00178

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

9. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry chalcone reductase and its use.

10. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry protein kinase and its use.

11. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry auxin-related gene and its use.

12. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry sucrose transporter and its use.

13. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the coding region or a fragment thereof, of a strawberry meristem pattern gene and its use.

14. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein with homology to transcribed sequence accession number T45086 and its use.

15. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein with homology to transcribed sequence accession number L36159 and its use.

16. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein with homology to transcribed sequence accession number T45902 and its use.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 97/ 00178

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

### 17. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence A and its use.

### 18. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence B and its use.

### 19. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence C and its use.

### 20. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence D and its use.

### 21. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence E and its use.

### 22. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence F and its use.

### 23. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence G and its use.

### 24. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence H and its use.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 97/ 00178

## FURTHER INFORMATION CONTINUED FROM PCT/SA/210

### 25. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence I and its use.

### 26. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence J and its use.

### 27. Claims 1-10 (partially):

A vector for use in genetic transformation of strawberry cells, comprising a promoter sequence, a regulation sequence and a transcription termination sequence, in which the regulation sequence comprises the sequence or a fragment thereof, selected from a strawberry protein of unknown homology encoded by the StrawRipe Sequence K and its use.

# INTERNATIONAL SEARCH REPORT

Information on patent family members:

International Application No

PCT/GB 97/00178

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